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HAMILTON B. G. ROBINSON

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DECEMBER, 1954

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1955 MEETING
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EDITOR'S VIEWPOINT

Jensen and Danø (p. 741) present an interesting study of crystallography of dental calculus. Renewed interest in calculus is commendable. The subject has been investigated from various approaches—physical, chemical, bacteriologic, and histologic. The use of crystallography, as the authors suggest, offers opportunity for new approaches. By means of the correspondence between our editorial consultant and the senior author, which we monitored, we learned that there are still differences of opinion in this field.

Dewar and Parfitt (p. 751), applying the technics outlined in their previous paper, were unable to find any correlation between salivary mucin, viscosity, ropiness, tack, and dental caries activity (as measured by clinical, biochemical, and bacteriologic methods). This finding is contrary to long-held impressions of many who have discussed but not tested some of these properties.

Haskins, Haack, and Ireland (p. 757) studied stress patterns in Class II cavities. They found that a greater depth of filling material led to better stress patterns, that rounding and sloping the axial wall did not consistently reduce stress, and that slight rounding of the pulpal-axial line angle had little effect in reducing stress. The latter procedure, however, is recommended by the authors for other reasons. The utilization of the methods employed in other fields for study of stress in dental restorations promises to improve our operative technics.

Wainwright (p. 767) determined the time required for radioactive-tagged nicotinamide, urea, thiourea, and acetamide to penetrate enamel of extracted teeth. The nicotinamide was relatively slow, but urea, thiourea, and acetamide penetrated one-half the thickness of the enamel within 20 to 34 minutes, and in longer periods all four substances entered the dentin. The rapid penetration of enamel by urea had been previously demonstrated (Wainwright and Lemoine). The possibilities of using radioactive carbon-tagged compounds as research tools and the use of penetrating compounds in therapy are self-evident.

Fleming and Greenfield (p. 780), by injections and feeding of high concentrations in water, forced fluorides across the placental barrier in mice. The teeth showed retardation in enamel mineralization, cartilage in the pulp, and vascular changes in the pulp. The periodontal bone showed cartilage. Interference with calcification of enamel is in accord with general concepts of fluoride effects, but the appearance of cartilage in areas of dentin and the altered periodontal bone formation suggest a metaplasia. It is proposed that fluorine, acting as an antimetabolic, interferes with the glycolytic cycle and thus with the high-energy phosphate necessary for calcification.

Radioactive fluoride has given some information on the uptake of fluorides, but its short half-life (112 hours) has limited its usefulness. Wallace-Durbin (p. 789), using a newer type counter, was able to obtain additional information on F metabolism. She found cell membranes freely permeable to NaF, no significant soft tissue deposits except in the kidney, no level of F intake below which excretion was complete, no difference in intravenously administered and ingested F, and no effect of age on skeletal uptake of F but a definitely lower F uptake in mature rat molars. With further improvement of technics additional information will be accumulated toward a more complete understanding.

Neumann has been a strong proponent of the theory that function is an important factor in caries' etiology. He and Di Salvo (p. 801) examined the exchange of radioactive phosphorus in the teeth of nonfunctioning and functioning halves of monkey mandibles. They found less radioactive phosphorus exchange on the functioning sides. They conclude that the exchange depends on "biologic variables" and that function may affect ionic balance and turnover rate. From these data we fail to see any support for the concept that nonfunction is an important physiologic factor in caries.

Savara and Suher (p. 808) give the data they obtained on carious prevalence in chil-

dren from 1 to 6 years of age. Although comparable information is frequently published on school-age children, data from younger children are not abundant. A marked increase in the percentage of children with caries and in the number of def teeth per child is seen between 2 and 3 years of age. This, the authors propose, indicates an increased susceptibility of the oral cavity to dental caries with the eruption of the primary molars. Perhaps there is no increase in susceptibility of the oral cavity but only more targets for an existing process. The need for extraction of one or more first permanent molars in 29.7 per cent at age 6 is striking. The authors compare their data with those of other recent surveys.

Kamrin (p. 824), extending his studies of caries in parabionts, presents evidence strongly supporting the view that the cariogenic action of carbohydrates is local. These artificially produced "siamese twin" rats had a common blood supply with equal sugar level within one hour of feeding. The animal given dextrose developed a high incidence of caries while the parabiont had little or no caries. Perhaps this technic can be successfully carried out on other animals, such as hamsters.

Keyes (p. 830) found surprising caries resistance in hamsters fed a diet presumably cariogenic. Variability in caries susceptibility in hamsters has appeared from time to time and is confusing. Certainly, the demonstration of Hunt, Hoppert, and Erwin of the inheritance of caries resistance in rats should sound a warning to others working with experimental animals. Unless standardized animals (in regard to caries resistance) are used, one must view with extreme caution results obtained on relatively small groups of laboratory animals.

Shafer and Muhler (p. 842) present some puzzling results of caries studies, fluorine metabolism, and salivary gland effects in rats with alterations in gonads and sex hormones. Orchiectomized rats showed lowered caries activity while ovariectomized rats showed decrease in one instance and increase in another. In general, diethylstilbesterol and estradiol seemed to increase caries. These hormones also increased fluoride concentration in bone, a somewhat parodoxical finding. The salivary gland changes could not be related to caries. Unevaluated genetic factors may have dominated the hormonal changes in these small groups of 18 animals each, or the various changes may have no causal relationship. These findings cannot be ignored, but conclusions at this stage do not appear to be warranted.

The measurement of the pH of minute intraoral areas (plaques, cavities) is of great importance in many investigations. The pros and cons of antimony electrodes have been argued. Brudevold and Thompson (p. 849 and p. 854, in two papers, describe an antimony electrode suitable for measurements within small regions and evaluate the instrument. Antimony electrodes appear to be less reliable than glass electrodes but their small size makes them desirable despite this shortcoming. Care should be taken to make comparisons between antimony-electrode readings rather than to take the pH's obtained as absolute.

Using radioactive labeled bicarbonate, Greulich and LeBlond (p. 859) studied the development of dentin. Within 4 hours after injection the bicarbonate appeared in the predentin adjacent to the odontoblasts. Within 24 hours this labeled predentin layer was changing into dentinal matrix. The growth value appeared more rapid in the first 10 days of life. The authors' careful study is a contribution to the understanding of odontogenesis. The stability of the dentinal matrix is emphasized. It is suggested, but not concluded, that mucopolysaccharides may be essential for deposition of dentin crystals.

The Section Nd of the American Association for the Advancement of Science met on Tuesday, December 28, on the campus of the University of California, San Francisco, California. The program included a symposium on "Growth and Development," a paper on "Stannous Fluoride in a Dentifrice as an Anti-Cariogenic Agent," and a symposium on "Radiation Hazards in the Dental Office." This is the first meeting of dentistry as a section of the A.A.A.S.

—H. B. G. R.

CRYSTALLOGRAPHY OF DENTAL CALCULUS AND THE PRECIPITATION OF CERTAIN CALCIUM PHOSPHATES

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INTRODUCTION

IT HAS previously been found by the authors[1] that calculi from the salivary glands frequently consist of the otherwise rare calcium phosphate whitlockite, or of whitlockite mixed with apatite.* The expected compound is apatite (possibly with a small admixture of calcite, as occasionally found in salivary calculus[1]), since apatite is the compound which always precipitates from dilute aqueous solutions in vitro at body temperature and within the pH range prevailing in the saliva. Apatite is also expected because it is the calcium phosphate of enamel, dentin, bone, and many pathologic concretions of the body. These findings were the basis for the hypothesis that saliva often, perhaps always, contains a factor impeding the precipitation of apatite. If this hypothesis is true we shall expect the calcium phosphates precipitated in saliva under normal conditions (dental calculus) to be as rich in whitlockite as are the pathologic calculi from the salivary glands. We shall perhaps expect an even higher whitlockite frequency in dental calculus, for some formation of calculi in the salivary glands may well be due to absence of the postulated factor impeding precipitation. As will be shown in the following, whitlockite is even more abundant in dental calculus than expected. It was found in 80 per cent of the 52 specimens examined. The percentage of calculi from the salivary glands which contained whitlockite was significantly smaller (54 per cent). Hence the hypothesis is substantiated.

PREVIOUS CRYSTALLOGRAPHIC STUDIES OF DENTAL CALCULUS

Papers on clinical, bacteriologic, and histologic aspects of calculus are abundant. Urbantschitsch[2] reviewed 53 papers, all earlier than 1933. The crystallographic literature, on the other hand, is meager.

Angel (in Urbantschitsch[2]) examined thin sections of dental calculus under the petrographic microscope. He found very small amounts of aragonite ($CaCO_3$) and larger amounts of a crystalline phosphate which he calls "metabrushite?" and of an amorphous phosphate which he calls "collophane?" Metabrushite is not a distinct mineral species but in general a partly dehydrated *brushite* ($CaHPO_4.2M_2O$)[3]; collophane is simply a colloidal apatite.[4] Philipp[5] and Mathis[6] (apparently different publications on the same experimental material, which is more detailed in Mathis' paper) examined calculus from a large but unspecified number of cases by x-ray crystallography. They found the specimens to give an apatite x-ray diagram in all cases except two: one was brushite and one whitlockite. One specimen of dental calculus was casually mentioned by Brandenberger,[7] who found it to be apatite.

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*Throughout this paper "apatite" is not used to signify any member of the apatite group but only a compound which gives an x-ray diagram similar to hydroxylapatite ($Ca_4(PO_4)_3OH$).

These early results seem to contradict our hypothesis. However, the mineralogic constitution of calculus in Danish individuals might differ from that of a Viennese population (Philipp,[5] Mathis[6]). The Viennese material might not have been so very large and, above all, Philipp and Mathis might, because of their comparatively unrefined x-ray technic (57 mm. camera diameter and copper radiation), have overlooked considerable admixtures and have classified mixed calculus as pure apatite in a number of cases.

MATERIAL

The 52 specimens of dental calculus examined were from 39 patients in a Danish urban population. They belong, however, to a group which voluntarily sees a dentist, a possibly significant restriction in sampling. Sixteen (labeled "G. P." in Table I) were from a general practioner among a population which might be described as upper middle class. Thirty-six (marked "R. C." in Table I) were obtained from the Royal Dental College, Copenhagen. No steps were taken to ensure a random sampling. We have been unable to reasonably define a random sampling of dental calculus among a given population. Many patients have no calculus while many have not enough for easy collection and examination.

Specimens in our material were collected only from patients who had rather more calculus than the few milligrams necessary for a powder diagram in our technic, since the methods using the petrographic microscope, which consume only tenths of a milligram are inadequate.[1] In general, subgingival and supragingival material was collected separately where possible. Materials from mandibular and parotid regions in the same patient were never mixed. The specimens as received were labeled with patient numbers and mouth region and were accompanied by occasional clinical notes. Although we have drawn one or two conclusions from the clinical notes, they will, for the sake of brevity, not be given.

TECHNIC

Calculus specimens were shaken with 2 or 3 portions of distilled water. Adhering mucous substance, blood etc., became suspended in the water and were discarded with it. Samples were dried and powdered in an agate mortar. The samples were almost always used up in making a powder specimen for the x-ray diagram. The x-ray technic was the same as employed in our previous investigation.[1] Particle sizes were not estimated. The relative abundance of whitlockite and apatite was estimated by comparing the calculus diagrams with a series of new diagrams of mixtures of synthetic whitlockite and apatite in the following weight proportions Wh :Ap = 0 :10, 1 :9, 3 :7, 5 :5, 7 :3, 9 :1, and 10 :0.

This enables the relative abundance of the 2 compounds to be fixed between 2 limits in any specimen. The method gives little indication of the absolute abundance, and it should be noted that the comparison diagrams were of synthetic phosphates of a high degree of perfection. The synthetic whitlockite and apatite were prepared by sintering stoichiometric proportions of calcium carbonate and pure synthetic brushite together. Optimal sintering

conditions (time, temperature, and atmosphere) were found in a series of experiments in which the conditions were varied and line width controlled photographically. The reflective power was simultaneous measured on the General Electric x-ray spectrometer. These experiments will be published elsewhere. From the method it follows that the quantitative results may be subject to error if the reflective power of imperfectly crystalline specimens of calculus apatite, viz., whitlockite, is unequally decreased from that of perfectly crystalline specimens of the 2 compounds. This error is inherent in any quantitative work with imperfectly crystalline substances, and it seems impossible to overcome it with only the few milligrams of mixture specimen at the investigator's disposal. The following conclusions, however, are valid irrespective of such errors in the abundance ratios found.

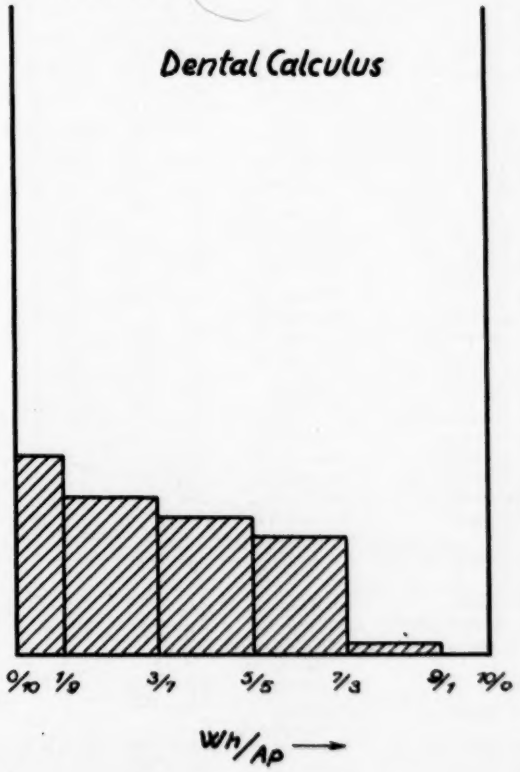

Fig. 1.

CALCIUM PHOSPHATES IN DENTAL CALCULUS

All results are summarized in Table 1 and the abundance ratio (apatite: whitlockite) in Fig. 1. The abundance ratio for our earlier material of calculi from the salivary glands is given in Fig. 2.* In calculi from the salivary glands 54 per cent were found to contain whitlockite; in specimens of dental calculus, 80 per cent. This is the most important result of our investigation

*New and more accurate determinations than those published previously.[1]

TABLE I

DESCRIPTION AND CONSTITUTION OF 52 SAMPLES OF DENTAL CALCULUS

NO.	ORIGIN	REGION*	RELATION TO GINGIVAL MARGIN	CONTAINS†	WH./AP.‡
1	R. C.			Ap. + Wh.	3/7-5/5
2	R. C.	Mandib. incis.	Supraging.	Ap. + Br. (30%)	0/10-1/9
3	R. C.	Left mandib. molars	Subging.	Ap. + Wh.	5/5-7/3
4	R. C.	Mandib. incis.	Supraging.	Ap. + Wh.	5/5-7/3
5	G. P.	Mandib. incis.	Supraging.	Ap. + Br. (60%)	0/10-1/9
6	G. P.	$\overline{4}$ ling.	Subging.	Ap. + Wh.	5/5-7/3
7	G. P.	Mandib. incis.	Supraging.	Ap. + Wh.	3/7-5/5
8	G. P.	Mandib.	Supraging.	Ap. + Wh. + Amalg.	1/9-3/7
9	G. P.	Maxill. molars	Supraging.	Ap. + Wh.	1/9-3/7
10	G. P.	Mandib. incis.	Supraging.	Ap. + Wh.	1/9-3/7
11	G. P.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
12	G. P.	Mandib. incis.	Subging.	Ap. + Wh.	3/7-5/5
13	R. C.	Maxill. and mandib. incis.	Subging.	Ap. + Wh.	3/7-5/5
14	R. C.	Mandib. incis.	Subging.	Ap. + Wh.	7/3-9/1
15	R. C.	Mandib. incis.	Supraging.	Ap. + Wh.	3/7-5/5
16	R. C.	Mandib. incis.	Supraging.	Ap. + Wh.	1/9-3/7
17	R. C.		Supraging.	Ap. + Wh.	1/9-3/7
18	R. C.		Subging.	Ap. + Wh.	3/7-5/5
19	R. C.	Mandib. incis.	Supraging.	Ap. + Wh. + Br. (60%)	1/9-3/7
20	R. C.	$\overline{4}$ ling.	Supraging.	Ap. + Wh.	5/5-7/3
21	R. C.	$\overline{43}$ ling.	Supraging.	Ap. + Wh.	3/7-5/5
22	R. C.	$\overline{3}$ ling.	Supraging.	Ap. + Wh.	1/9-3/7
23	R. C.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
24	R. C.	Maxill. incis.	Subging.	Ap. + Wh.	3/7-5/5
25	R. C.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
26	R. C.	$\overline{1}$ ling.	Supraging.	Ap. + Wh.	3/7-5/5
27	R. C.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
28	R. C.	Maxill. premolars	Supraging.	Ap. + Wh.	3/7-5/5
29	R. C.	Mandib. incis.	Supraging.	Ap. + Wh.	1/9-3/7
30	R. C.	Mandib. incis.	Subging.	Ap. + Wh.	5/5-7/3
31	R. C.	Maxill. incis.	Subging.	Ap. + Wh.	1/9-3/7
32	R. C.	Mandib. incis., facial.	Supraging.	Ap. + Wh. + Br. (30%) + CaOx	3/7-5/5
33	G. P.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
34	G. P.	Mandib. incis.	Supraging.	Ap. + Br. (60%)	0/10-1/9
35	G. P.	Mandib. incis.	Supraging.	Ap. + Wh.	1/9-3/7
36	G. P.	Mandib. incis.	Supraging.	Ap. + Wh.	3/7-5/5
37	G. P.	Mandib. incis.	Supraging. + subging.	Ap. + Wh.	1/9-3/7
38	G. P.	Mandib. incis.	Supraging.	Ap.	0/10-1/9
39	G. P.	$\overline{1}\vert\overline{1}$		Ap.	0/10-1/9
40	G. P.	Mandib. incis.	Supraging.	Ap. + Wh. + CaOx	3/7-5/5
41	G. P.	Maxill. molars, facial	Supraging.	Ap. + Wh. + Amalg.	5/5-7/3
42	R. C.	Mandib. incis.	Subging. + supraging.	Ap. + Wh.	5/5-7/3
43	R. C.	$\vert 6$	Supraging.	Ap. + Wh. + CaOx	5/5-7/3
44	R. C.	$\overline{\text{Mandib. incis.}}$	Subging. + supraging.	Ap. + Wh.	3/7-5/5
45	R. C.	Mandib. incis.	Supraging.	Ap. + Wh. + Br. (30%)	1/9-3/7
46	R. C.	Mandib. incis.	Supraging.	Ap. + Wh. + Br. (30%) + CaO	1/9-3/7
47	R. C.	Mandib. incis., facial and lingual	Supraging.	Ap. + Wh.	1/9-3/7
48	R. C.	$\vert 456$	Supraging.	Ap. + Wh. + CaOx	1/9-3/7
49	R. C.	$\overline{321}\vert$ facial and lingual	Supraging.	Ap.	0/10-1/9
50	R. C.	$6\vert$	Supraging.	Ap. + Wh.	1/9-3/7
51	R. C.	$\overline{\text{Mandib. incis.}}$, facial and lingual	Subging.	Ap. + Wh.	3/7-5/5
52	R. C.	Mandib. incis.	Supraging.	Ap. + Wh.	1/9-3/7

*Lingual surfaces unless otherwise indicated.

†Ap. = apatite, Wh. = whitlockite, Br. = brushite, Amalg. = dental amalgam, CaOx = $CaOx \cdot H_2O$. The amount of brushite is roughly estimated in per cent of the total.

‡The limits for the value of whitlockite/apatite are estimated by means of the standard mixtures named previously.

and substantiates our hypothesis of a factor in the saliva which impedes the precipitation of apatite. The whitlockite frequency is significantly higher in dental calculus than in salivary calculi. This might indicate that the apatite-impeding factor, while not necessarily a specific compound, is sometimes low or absent in the saliva of patients with salivary calculi. Several other explanations for the difference are possible.

It was noted that calculus whitlockite was almost always more crystalline than was calculus apatite (that is, its powder diagram lines showed less broadening).

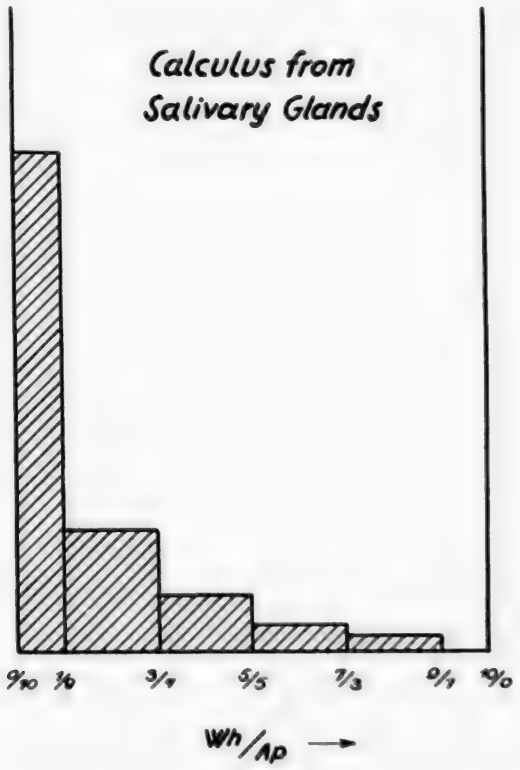

Fig. 2.

Brushite was found in 7 of 52 specimens. These 3 compounds (apatite, whitlockite, and brushite) are the same as those found in dental calculus by Urbantschitsch and Angel,[2] Philipp,[5] and Mathis.[6] But whereas Philipp and Mathis sought in vain for relationships between mineralogic constitution and other properties (color, etc.), in dental calculus, our material demonstrates several such relations.

1. Brushite is found only on mandibular anterior teeth. Seven samples of 22 specimens of mandibular anterior calculus contained brushite. Thirty specimens of calculus from other regions contained no brushite. Several of the 7 mandibular samples were from patients denoted with "calculus abun-

dant.'' This remark was never made about patients without brushite. Supersaturation with brushite requires a higher pH than supersaturation with apatite at constant calcium and phosphate concentration (Bjerrum[8]). Schmidt-Nielsen[9] and Ericsson[10] have shown that pH is almost always higher in mandibular than in parotid saliva and hence the conditions for calculus formation are more favorable. The finding of brushite in mandibular calculus is what one might expect. Schmidt-Nielsen[9] and Ericsson,[10] being chiefly interested in enamel solubility, calculated the degree of saturation with respect to apatite for a large number of analyses of saliva. From the point of view of calculus formation, brushite is also of interest, so we have calculated from the data of Schmidt-Nielsen[9] the degree of saturation n for brushite in the saliva of a student group.

Since we have incongruous solutions, n_{brush} is defined as $n_{brush} = \sqrt{\frac{I}{L}}$

Where I is the ionic product of brushite and L its solubility product. The value of L employed is the one determined by Bjerrum.[8] The values obtained are given in Table II.

TABLE II

DEGREE OF BRUSHITE SATURATION IN THE SALIVA OF 19 STUDENTS

REGION	A	B	C	D	E	F	G	H	I	J
Mandibular region	1.34	1.04	1.15	1.34	1.07	1.91	1.40	1.41	1.31	1.62
Parotid region	0.82	0.37	0.30	0.73	0.51	0.64	0.54	0.74	0.62	0.66
	K	L	M	N	O	P	Q	R	S	
Mandibular region	0.76	0.96	1.33	1.50	1.06	1.37	0.79	1.09	1.21	
Parotid region	0.48	1.05	0.62	0.51	0.49	0.62		0.60	0.50	

It is evident that the saliva in the parotid region is not saturated with brushite, while the mandibular saliva in all but 3 cases is supersaturated. This agrees well with the finding of brushite in mandibular calculus only. It will be remembered that brushite was found in only 7 samples of mandibular calculus out of a total of 22 specimens examined. This seems quite natural, for if the concentration of calcium or phosphate or the pH in mandibular saliva temporarily decreases brushite will dissolve before apatite because the saliva will become unsaturated with brushite before apatite. Moreover, because brushite is a soft crystal and apatite a hard one, brushite dissolves much faster than does apatite. Brushite calculus, by going into solution, probably prevents the saliva from becoming unsaturated with apatite.

2. Whitlockite is more abundant in subgingival than in supragingival calculus. The average abundance ratio Wh./ap. was 46:54 in the subgingival material against 26:74 in the comparable supragingival material. Specimens from anterior mandibular teeth were not included in either group.

3. From one patient calculus specimens were obtained from 4 different places in the parotid region (Nos. 20 to 23). The higher the index, the more whitlockite was found. Too much weight should not be placed on a result from one patient, but it does not seem to be a pure accident, and it fits into our general picture.

Whitlockite, then, is deposited where n_{ap}, the supersaturation with respect to apatite, is low. The n_{ap} is low in the subgingival area because of acid-producing fermentation. In the parotid region n_{ap} is low because parotid saliva is not very supersaturated with apatite.

It remains to be seen whether the reason for Whitlockite being preponderant is that the apatite-inhibiting factor is more active at low supersaturation, or that the better crystalline whitlockite (vide infra) is more resistant to redissolution by acid from periodic bacterial action than poorly crystalline apatite. Both hypotheses seem a priori plausible.

CORROSION PRODUCTS OF DENTAL AMALGAM FOUND IN CALCULUS SPECIMENS

One diagram (No. 8) contained a series of sharp, clear lines not due to any expected substance. The d-values (interplanar spacings) were calculated for all lines in the diagram which were not due to apatite. By means of the d-values for the 3 strongest lines, and without any further assumptions, a substance was identified by means of the A.S.T.M. card index of powder diagrams. The substance was the mineral moschellandsbergite[11] Ag_2Hg_3 or Ag_5Hg_8 or $Ag_{10}Hg_{13}$, better known as the γ_1 phase of the silver-mercury alloy system, and a major constituent of dental amalgam. The result was somewhat unexpected since the calculus specimen was of a light color. Later, apparently the same system of lines was found in the diagram of Specimen 41.

Not all nonphosphate lines present were due to Ag_2Hg_3. An attempt was made to identify all the lines by consulting the literature on the metallography and crystallography of dental amalgams.[12-16] The data for nonphosphate lines on Films 8 and 41 were compared with the data from a diagram of an amalgam filling prepared lege artis and by the dentist who had made the fillings for No. 41. This filling was prepared in the cavity of an extracted tooth, kept under water at 37° for 2 weeks, after which it was filed, and a diagram was taken from filings of the hardened filling. All the lines in Diagrams 8 and 41 not due to calculus phosphates were found to be due to components of set amalgam. We had hoped to find out whether all components corrode equally fast at the surface of an amalgam filling in the oral cavity. We are not aware of any previous attempt to answer this question. The material was insufficient to provide a definite answer.

TABLE III

CRYSTALLINE PHASES FOUND IN DENTAL AMALGAMS

NAME AND COMPOSITION	CRYSTAL SYSTEM	AXIAL LENGTHS (T. J. AND M. D.)
$\gamma_1 = Ag_5Hg_8$	Cubic	$a = 10.032$Å
δ_1 = solid solution of Hg in Ag	Hexagonal	($a \approx 2.99$Å; $c \approx 4{:}83$Å)
γ_2 = solid solution of Hg in Sn	Hexagonal	$a = 3.209$Å; $c = 2.984$Å
Ag_3Sn (the unattacked "alloy")	Hexagonal	$a = 3.001$Å; $c = 4.816$Å

The diagram of our pure amalgam contained 56 lines. Forty-two were due to $\gamma_1 = Ag_5Hg_8$,10 to $\gamma_2 =$ Hg in Sn, 9 to $Ag_3Sn =$ "alloy." Five lines were superpositions of lines from 2 phases, and no unidentifiable lines were

present. A small amount of β_1 = Hg in Ag may also have been present. This is difficult to detect. The 3 strongest lines of β_1 "clash" with 2 rather strong lines of Ag_3Sn and 1 very strong γ_1 line.

The axial lengths were found to be somewhat different from values quoted in literature. The axial lengths need not by necessity be constant, for the phases are solid solutions. Our values, however, were the same within experimental error in all 3 specimens. They may then be nearer to the normal values for set amalgam than those of Gayler[12] and Troiano.[13] The relative intensities of the lines from amalgam compounds in Diagrams 8 and 41 and of the intact filling indicate a difference in relative abundance of the several phases. The lines from Ag_3Sn = "alloy" were very weak in 8 and 41, indicating a much lower percentage of "alloy" in the corroded than in the intact amalgam. In the same way it was found that the corroded amalgam in 41 had a somewhat lower percentage of γ_2 = Hg in Sn than had the corroded amalgam of 8 and the intact filling.

Had the whole fillings of 8 and 41 been at our disposal for comparison, we should have been able to say with certainty whether this was so because Ag_3Sn and γ_2 are less resistant to corrosion than the main constituent of a filling $\gamma_1 = Ag_5Hg_8$. As it is, it can only be stated that we believe it is the case with Ag_3Sn, and that the methods of x-ray crystallography are capable of proving or disproving it, once the materials (corrosion product and underlying filling) are available.

MINOR CRYSTALLINE CONSTITUENTS OF CALCULUS

On diagrams of 5 calculus samples (Nos. 32, 40, 43, 46, 48), a few extremely weak lines due neither to the calculus phosphates nor to the amalgam phases previously dealt with were found. Minor quantities of other compounds must have been present. By aid of the A.S.T.M. card index it was found that 2 of these lines agreed with the 2 strongest lines of calcium oxalate-monohydrate, a compound not unlikely to precipitate occasionally in the oral cavity. A third fairly strong line in the $CaOx{\cdot}H_2O$ diagram is situated at about $\theta = 8.63°$. This line was not found in our diagrams. At low θ values the diagrams have a high background, so it is very difficult to see extremely weak lines.

TABLE IV

COMPARISON OF THE GLANCING ANGLE θ FOR THE 3 UNKNOWN LINES FROM CALCULUS 32, 40, 43, 46, AND 48 WITH THE 3 STRONGEST LINES OF $CaOx{\cdot}H_2O$. COBALT Kα RADIATION

θ	32	40	43	46	48	$CaOx{\cdot}H_2O$
∽ 9°						8.63°
∽ 9°		9.32°		9.32°	9.35°	
∽14°	14.12°	14.12°	14.14°	14.21°	14.20°	14.16°
∽17°	*	17.67°	17.67°	*	17.65°	17.54°

*The line at θ ∽ 17° could be seen on these films but it was impossible to measure its position because of interfering lines from other compounds.

The agreement of θ values for the lines at θ ∽ 14° and θ ∽ 17° makes it very likely that $CaOx{\cdot}H_2O$ has been present. A line in our diagrams situated at $\theta = 9.32° - 9.35°$ is definitely not from $CaOx \cdot H_2O$, but from a compound so far unidentified.

CONCLUDING OBSERVATIONS

The object of this study was to provide an orientation in the crystallography of dental calculus and a check on the authors' hypothesis of an apatite-impeding factor in the saliva. More extensive studies along similar lines might well be profitable. In any such study, full clinical observations (e.g., regarding viscosity of the saliva and age of calculus) should be made and utilized. Specimens from different regions should always be kept separate, and they should be examined under the microscope and described before powdering for x-ray analysis.

Surveys in different populations might be worth making. Similar surveys of urinary calculus (literature in Prien,[17]) in different populations show large differences of frequency for different calculus types. These differences are to a large extent nutritionally conditioned, but they are not yet fully understood. Similar differences in dental calculus should perhaps be expected.

After the conclusion of this study 2 extremely interesting papers by Masao Kusunoki came to our attention.[18, 19] In the former Kusunoki demonstrated that patients with dental calculus in general have a saliva of low viscosity and low protein content. In the latter he showed that the higher the mucin content in a saliva the lower the chance for calculus formation, from which he concluded that mucin inhibits the formation of calculus. This is the only mention of a calculus-inhibiting factor in the saliva which we have found in the literature. If Kusunoki's anticalculus factor is identical with our anti-apatite factor arrived at by a different route, calculus from patients with saliva of high viscosity should be richer in whitlockite than calculus from patients with saliva of low viscosity. Kusunoki also determined phosphate calcium and pH in the saliva of his patients. Unfortunately, he did not determine conductivity or calculate degrees of saturation, as did Schmidt-Nielsen[9] and Ericsson.[10] He denied any correlation between calculus formation and pH, or phosphate, or calcium concentration. We, on the other hand, should think that the degree of saturation would be at least loosely correlated to calculus frequency, because we have shown previously that there is a clear correlation between degree of saturation and formation of brushite calculus.

SUMMARY

Fifty-two samples of dental calculus were examined by x-ray crystallography. They consist almost exclusively of mixtures of 3 calcium phosphates—colloidal apatite, colloidal whitlockite (somewhat less dispersed than the apatite), and macrocrystalline brushite.

Apatite was found in all samples. The otherwise very rare whitlockite was found in 41 of 52 specimens, a higher frequency of whitlockite than that found in calculi from the salivary glands. This substantiates the hypothesis put forward by the authors, that there is present in saliva a factor which impedes the precipitation of apatite. Brushite was found in 7 samples of 21 in calculus from the mandibular anterior teeth but not in other regions of the mouth. A recalculation of Schmidt-Nielsen's analyses[9] of saliva from 19 students showed that their mandibular saliva had been supersaturated with brushite in all cases but three, while their parotid saliva had been unsaturated

in all cases but one. Corrosion products of amalgam fillings were found in 2 calculus samples. The composition of these corroded fillings was compared with that of a freshly prepared filling. The comparison seems to indicate that Ag_3Sn "alloy" in the oral cavity dissolves faster than the other phases present in set amalgams. The corroded surface and the underlying intact filling from the same tooth should, however, be investigated in order to give a final answer to the question of corrosion velocity of different phases in amalgam fillings.

Five of the samples contained small amounts of a crystalline compound which is almost certain to be calcium oxalate-monohydrate. Three of the 5 samples contained minute quantities of another compound, so far not identified.

We are indebted to Drs. P. O. Pedersen, Gertrud Lund, and W. Riis Klausen for supplying the calculus material. We thank Drs. A. Unmack and Tso-Yueh Huang for their aid in making the diagrams. Carlsbergs Mindelegat, for Brygger J. C. Jacobsen, has financially supported a research program in the chemistry of calcium phosphates, of which the present study forms a part.

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MUCIN CONTENT, PHYSICAL PROPERTIES OF SALIVA AND CARIES ACTIVITY

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ALTHOUGH it is a common belief among dentists that ropy, viscous saliva is associated with a high susceptibility to dental caries, there is little authoritative evidence to support this point of view. Such quantitative measures as have been made to determine the nature of this relationship have assumed that these physical properties are due to a high mucin content. We have previously established a connection between the state of the mucin and the magnitude of these physical properties.[1] In other published reports, no correlation was found between the physical properties and caries activity by Lothrop and Gies,[2] Pickerill,[3] and Rae and Clegg.[4] Krasnow and Oblatt[5] and Karshan[6] have investigated protein content of the saliva in relation to dental caries and found no definite relationship. Willsmore[7] studied the osmotic pressure and viscosity of saliva and concluded that there was a relationship between the occurrence of gingival cavities and highly viscous saliva. The validity of most of these conclusions is questionable because of difficulty in evaluating the methods of collection, treatment, or testing of the salivary samples. Further complication is added by uncertainties which surround the estimations of the activity of caries.

Attempts to decide on theoretical grounds whether the physical properties of saliva (which in turn are closely correlated with the mucin content) will influence caries activity in any particular direction will almost certainly give invidious results. On one side it can be argued that an increased protein content in the saliva would provide increased plaque material,[8] a better substrate for acidogenic bacteria,[9-11] increased amino acids which might serve as a source of acid,[12] and an interference of proteolytic enzymes and lysozyme activity,[13] all factors which might predispose toward dental caries. On the other hand, it may be suggested that the increased protein content and concomitant increased viscosity would enable the saliva to hold more inorganic material in suspension and thereby increase its buffer capacity both as the result of increased inorganic as well as protein content. Obviously the only way in which anything like a satisfactory answer can be given to this problem is to investigate the relationship with more critical procedures than have been previously employed. This paper is a report of such an effort.

This paper is based on a portion of a thesis submitted to the University of London, England, in support of the senior author's candidature for the degree of Doctor of Philosophy.

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METHODS

Collection and Testing of Saliva.—Both resting and stimulated saliva were used. Resting saliva was collected by allowing saliva to run from the mouth into a test tube for ten minutes. The samples were immediately placed on ice. Stimulated saliva was obtained by chewing paraffin wax for periods of five and fifteen minutes. The samples were collected in the mornings of three different days at least two hours after any food or drink had been taken.

Samples of saliva from 140 individuals aged 4 to 35 years were tested. Resting saliva samples were used for measuring *spinnbarkeit* (the capacity to be drawn into a thread), tack, viscosity, and mucin content. The activated saliva samples were employed for caries activity tests.

Measurements of Physical Properties of Saliva.—It has been pointed out[1] that there are a number of measurements which can be made on saliva which have not previously been investigated in relation to caries activity. These are viscosity, tack, and *spinnbarkeit.* A discussion of the nature of these properties and of an account of methods of determining them has been given[1] and so will not be presented here further than to say that viscosity was measured by the time taken for a ball to fall through a confined column of saliva at standard pressure, *spinnbarkeit* was the height to which the saliva could be drawn, and tack the weight capable of being borne by the adhesion of saliva to a disc of known area. The mucin content of saliva was estimated by Congo red in acid alcohol (A.C.R.A.) titer and by measuring tyrosine present in hydrolyzed saliva.

Assessment of Caries Activity.—Obviously there is little point in applying improved methods of measuring the physical properties of saliva to the problem of the relationship to caries unless the best possible assessment of caries activity is made at the time of the investigation. There is no unanimity of opinion as to how caries activity can best be measured. The procedures employed are either tests carried out in the laboratory on samples of saliva or clinical examinations. We have endeavored to combine both.

Clinical examinations were carried out using a standard mirror and probe and, in the case of the low caries group, using x-ray films of interproximal areas. "Sticky" fissures in which there was a question of caries activity were recorded separately. Most of the patients examined could not be followed for a period of more than a few months, so only one clinical examination was made and caries activity was recorded as DMF teeth. In previous work,[14] one of us found that if standard conditions of collection and treatment were followed, three different laboratory tests gave an indication of caries susceptibility. These were the lactobacillus count,[15, 16] the buffer capacity of the saliva,[17] and the modification of Fosdick's chemical tests.[14] Each of these was found to be correlated statistically with caries activity as judged by the number of decayed, missing, and filled teeth. It has been found possible to combine these in one statistical expression of caries susceptibility, giving due weight to each test according to the degree of its particular correlation with

caries susceptibility. On the basis of the statistical analysis of the results obtained from the study of eighty-six individuals, an estimating equation was derived.

$$\widetilde{X}_0 = 11.00\,X_1 - 4.42\,X_2 - 5.83X_3 + 0.76$$

where $\widetilde{X}_0$ = caries activity as predicted from the results of the laboratory tests
X_1 = log lactobacillus count
X_2 = buffer capacity value
X_3 = modified Fosdick test value.

The application of this equation raised the correlation coefficient to 0.84 as against +0.79 for the log of the lactobacillus count, –0.58 for the buffer capacity of saliva, and –0.70 for the modified Fosdick test. Thereafter, this was combined with the clinical assessment by means of a formula $Y = \frac{X_0 + \widetilde{X}_0}{2}$ (X_0 = number of DMF teeth), and the findings were used to establish grades of susceptibility as follows:

when Y =	0- 4 =	±	immune
=	5- 8 =	+	slightly susceptible
=	9-16 =	++	moderately susceptible
=	17-28 =	+++	highly susceptible
>	28 - =	++++	rampant.

RESULTS

Plotting of the composite caries activity figure against each of the physical measurements made on saliva (Fig. 1) showed that no correlation was apparent between dental caries experience and the physical properties of saliva.

The coefficient of correlation of the caries susceptibility figure (Y) to A.C.R.A., to viscosity, to *spinnbarkeit*, and to tack was in all cases less than 0.1. The correlation coefficient between Y and the mucin content as measured by the tyrosine present was 0.19.

Tables I and II show numerically that no relationship exists between the physical properties of saliva and the mucin content and caries activity.

In the present investigation, the results were also analyzed for gingival caries to see if there was a correlation between the number of carious cavities occurring on the lingual and buccal surfaces of the teeth and saliva viscosity or *spinnbarkeit* (Table III).

Contrary to Willsmore's findings, no correlation was found. The correlation coefficients for gingival cavities to saliva viscosity and to saliva *spinnbarkeit* were less than 0.1 for the total number of individuals and also for those individuals of 17 years and under.

COMMENT

The inclusion of the bacteriologic and biochemical tests in this present investigation of the colloidal properties of saliva afforded, incidentally, confirmation of previous findings of the value of these tests in assessing caries

activity. The correlation coefficients of X_0 to $\widetilde{X}_0$ (clinical to laboratory assessment) were 0.84 for the eighty-six individuals in the first group tested and 0.78 for the seventy-five individuals of similar age group in the present collec-

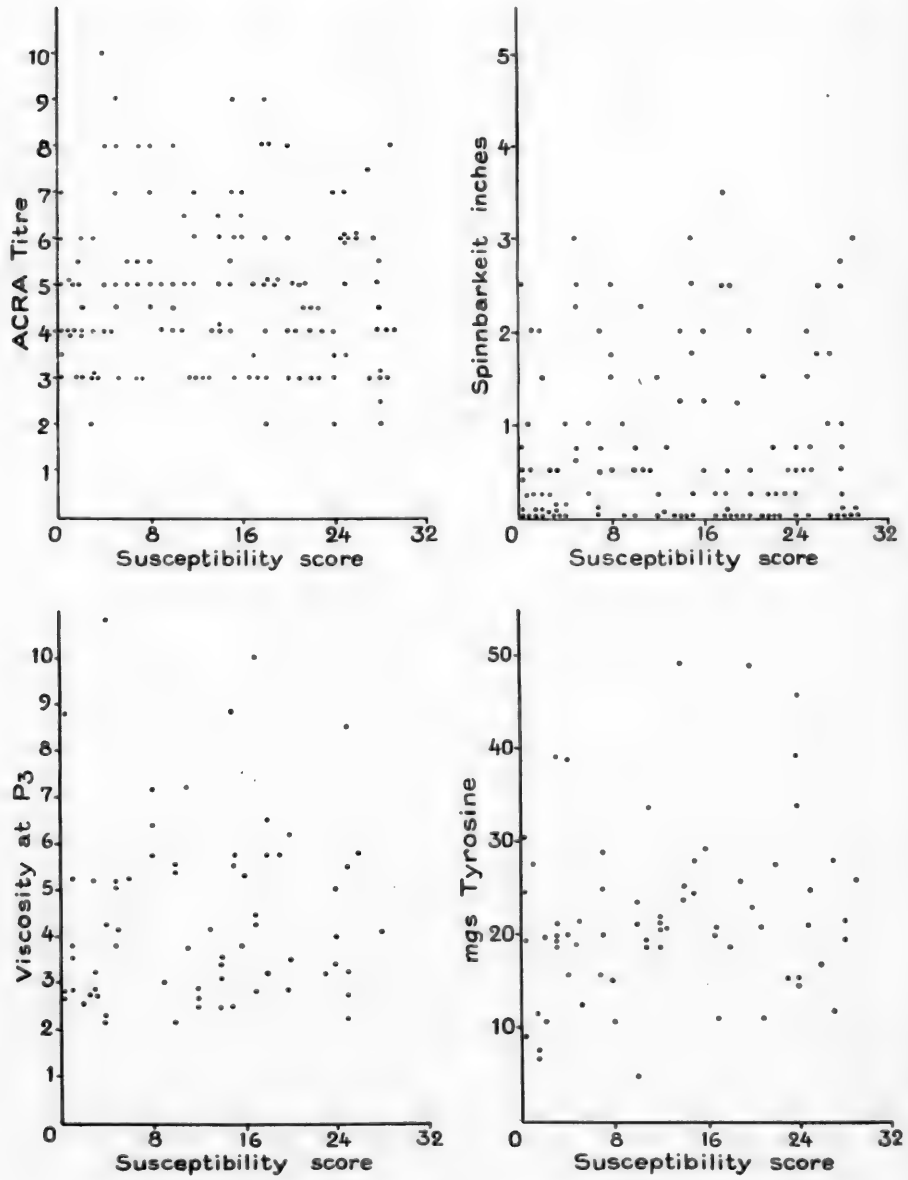

Fig. 1.—Correlation of salivary properties and caries activity.

tion. In the entire group of 140 individuals whose ages ranged from 4 to 35 years, the value dropped to 0.52. Such a drop was due to poor agreement among the older group, for in the sixty-five individuals over the age of 17, the correlation coefficient of X_0 to $\bar{X}_0$ was 0.48.

TABLE I
CORRELATION OF CARIES ACTIVITY WITH MUCIN CONTENT OF SALIVA

CARIES SUSCEPTIBILITY	MUCIN CONTENT AS SHOWN BY: A.C.R.A. TITER NO. CASES	A.C.R.A. TITER MEAN	A.C.R.A. TITER σ^*	TYROSINE (MG./100 ML.) NO. CASES	TYROSINE (MG./100 ML.) MEAN	TYROSINE (MG./100 ML.) σ^*
±	30	4.6	1.5	18	20.4	9.1
+	19	5.2	1.4	10	23.1	10.0
++	34	5.1	1.6	12	25.6	8.1
+++	43	5.2	1.7	24	25.8	10.3
++++	14	4.6	1.5	4	23.0	7.9

σ^* = Standard deviation = $\sqrt{\frac{\Sigma\ (M-r)}{n}}$

where M = mean
r = reading
n = number of cases.

TABLE II
CORRELATION OF CARIES ACTIVITY WITH PHYSICAL MEASUREMENTS ON SALIVA

CARIES SUSCEPTIBILITY	*Spinnbarkeit* (INCHES) NO. CASES	*Spinnbarkeit* (INCHES) MEAN	*Spinnbarkeit* (INCHES) σ	VISCOSITY (CENTIPOISES *at* P_3) NO. CASES	VISCOSITY (CENTIPOISES *at* P_3) MEAN	VISCOSITY (CENTIPOISES *at* P_3) σ	TACK (GM.) NO. CASES	TACK (GM.) MEAN	TACK (GM.) σ
±	30	0.6	1.1	15	4.13	1.72	30	5.08	0.16
+	19	0.9	0.9	11	4.46	1.36	14	5.11	0.17
++	29	0.7	0.7	20	4.12	1.44	25	4.90	0.12
+++	37	1.0	1.1	22	4.70	1.71	29	5.04	0.17
++++	14	0.8	1.1	3	3.50	0.58	5	4.95	0.21

TABLE III
INSPECTION OF RELATION OF GINGIVAL CARIES TO VISCOSITY AND SPINNBARKEIT

NO. PERSONS	GINGIVAL CAVITIES	AVERAGE NO. GINGIVAL CAVITIES	MEAN VISCOSITY AT P_3 (CENTIPOISES)	MEAN *Spinnbarkeit* (INCHES)
59	0	0	4.0	0.7
41	1- 3	1.9	5.3	1.0
23	4- 6	4.6	3.0	0.7
22	7-19	9.4	5.6	0.9

This lack of agreement among the older group may be due to a change with age in the influence of the various salivary factors on caries activity or to the unsuitability of the DMF figure assessment over the age of 17. It is well established, however, that the DMF figure is misleading as an index of concurrent caries activity in the older age groups, as the DMF figure includes clinical changes of many years' standing. So in these cases it seems justifiable to claim that the laboratory assessment of caries activity is more accurate than DMF value.

As DMF was considered in obtaining the susceptibility score (Y), it was felt that perhaps Y for the older group was open to criticism. Therefore, the

group of 17 years and under were considered separately for possible correlation of caries activity and mucin content of saliva and physical properties of saliva. Coefficients of correlation were from –0.1 to +0.1, which is no different from those of the complete group.

SUMMARY

Measurements of the several physical properties and the mucin content of saliva of 140 individuals 4 to 35 years of age were compared with their caries activity as estimated by a method which combined clinical examinations and laboratory tests.

No relationship was demonstrated between caries activity and either the mucin content of resting saliva as measured by tyrosine content and A.C.R.A. titer or the physical characteristics of saliva as demonstrated by the values for viscosity, *spinnbarkeit*, and tack.

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A STUDY OF STRESS PATTERN VARIATIONS IN CLASS II CAVITY RESTORATIONS AS A RESULT OF DIFFERENT CAVITY DESIGNS

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INTRODUCTION

A VARIETY of Class II cavity preparations were investigated to determine which cavity form would be most desirable for a silver amalgam restoration in a primary molar tooth.[1] It was found that side retention grooves placed in the proximal box portion of the cavity preparation and rounding and sloping the pulpal wall were the most beneficial in increasing the resistance of the silver amalgam restoration to fracture. Rounding the pulpal-axial line angle and placing a U-shaped retention groove in the gingival wall were found to be much less effective. However, the most interesting finding was that when all these principles were included in the same cavity design, the resistance of the silver amalgam restoration to fracture was increased far beyond the additive sum of the principles tested.

Following the previously mentioned investigation, another study suggested itself: to determine what effect different Class II cavity designs would have on the stresses in the restoration. It was with this idea in mind that a photoelastic study of various Class II cavity preparations was undertaken.

It would be very difficult, if not impossible, to study such a photoelastic model in three dimensions. Therefore, it was decided to study first several factors which could be seen in a mesiodistal cross section of a tooth. These factors were: (1) Occlusal steps of various depths, (a) shallow and (b) deep, (2) the vertical axial wall, (3) the rounding and sloping vertical wall, (4) the horizontal pulpal wall, (5) the rounding and sloping pulpal wall, (6) the rounding pulpal-axial line angle, and (7) the square pulpal-axial line angle.

Before beginning this project we realized that it would be a qualitative study and that the results obtained could not be used for quantitative measurement. The results, however, could be used on a comparative basis, one result compared with and against another result of this same study.

REVIEW OF THE LITERATURE

We were unable to find a single report of an investigation designed to study the internal stresses which are formed in the restoration when different principles of cavity preparation are employed. Noonan[2] reported recently on the

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use of photoelasticity in a study of cavity preparations, concluding that a flat cavity floor permits less stress concentration than one which is rounded, a rounded angle permits less stress concentration than a sharp angle, and retention points should not be sharp, but rounded to decrease stress concentration.

METHODS AND MATERIALS

For diffused field photoelastic work it is desirable to have a fairly large photoelastic model. Therefore, a mandibular second primary molar, ten times actual size, was selected and prepared for this study. Cold molding compound was used to make a synthetic rubber mold of the model.

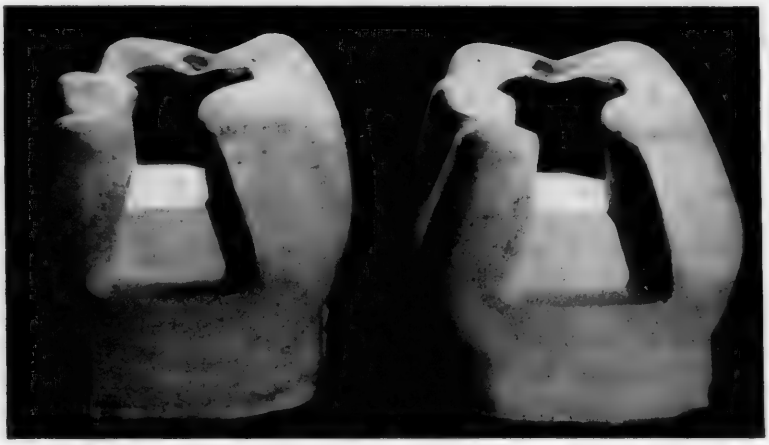

Fig. 1.—Model on left has square pulpal-axial line angle. Model on right has rounded pulpal-axial line angle.

A mesial-occlusal cavity was carved in a plaster tooth made from the rubber mold. Because the model was ten times the size of an average tooth, all cavity preparations were made ten times the dimensions one might employ for the average-sized tooth. Measuring from the deepest point of the occlusal surface, the pulpal wall was made 10 mm. deep. The mesiodistal width of the gingival seat was ten times the width of a No. 558 fissure bur. All walls and floors of the cavity were flat, and the pulpal-axial line angle was square.

A mold of the plaster model tooth, in which the mesial-occlusal cavity had been prepared, was made with the cold molding compound. A plaster of Paris model was secured from this mold, and the original cavity preparation was changed by lowering the pulpal wall 5 mm. A rubber mold was then made of this tooth and the cavity preparation.

Rubber molds were now available so that the three models could be duplicated.

Sixteen plaster models were required in order to study each cavity form in all possible combinations. Therefore, sixteen models were secured (eight of each basic cavity preparation), and the cavity modifications were added separately to the plaster models.

Details of Principles Tested.—

1. ***Rounded pulpal-axial line angle:*** The pulpal-axial line angle was evenly rounded on the appropriate cavities (Fig. 1).

2. ***Rounding and sloping the pulpal floor:*** The rounding was started at a point approximately beneath the central pit of the occlusal surface and extended to the pulpal-axial line angle. In the region of the central pit, the extent of the rounding was narrow and shallow with the cut gradually becoming wider and deeper until the pulpal-axial line angle was reached. At the pulpal-axial line angle, the cut was ten times one-half the diameter of a No. 4 round bur. In actual measurements this cut was 33.2 mm. long and 7.5 mm. deep at the pulpal-axial line angle (Fig. 2).

Fig. 2.—Model showing extent of rounding and sloping the pulpal wall of the cavity preparation.

3. ***Rounding and sloping the axial wall:*** To the best of our knowledge, this principle has never been advocated or employed for Class II cavity preparations. Sloping and rounding the axial wall was included in this investigation because engineering principles indicated that lower stress concentration at the pulpal-axial line angle should result from a structure of an elliptical form. The slope of the axial wall was started very shallow, 5 mm. above the gingival wall, and was gradually deepened as the cut was brought occlusally to the pulpal-axial line angle. At this point the cut was 7.5 mm. deep. The length of the cut on the axial walls varied because of the different depths of pulpal walls which were investigated (Fig. 3).

4. ***Shallow and deep occlusal steps:*** Pulpal wall depths of 10 mm. and 15 mm. were studied (Fig. 4).

Filling the Cavities.—Plaster indices of the occlusal and mesial surfaces of the original plaster models were made. The purposes of these indices were to help contour the filling material as it was poured into the cavities and to establish uniform occlusal and proximal thickness of the filling.

Prior to filling the cavities the sixteen plaster models and the mesial and occlusal indices were given three separate coats of plastic model spray. When the plastic spray was dry, the models and indices were covered with a thin

coating of petrolatum. The cavities in the models were filled separately with plaster of Paris which had been colored red so that the filling could be distinguished from the tooth.

Fig. 3.—Model showing extent of rounding and sloping the axial wall of the cavity preparation.

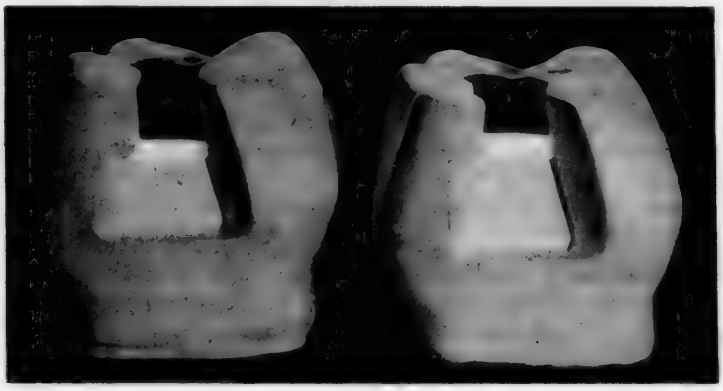

Fig. 4.—Models showing cavity preparations with pulpal wall depths of 15 mm. (*left*) and 10 mm. (*right*).

Preparing the Plaster Filling Model.—The mesial and distal pits on the occlusal surface of the plaster teeth were used as guide points, and the sixteen models each were cut into two halves with an electric band saw. The inside surfaces of all buccal portions of the teeth were smoothed with an electric sanding machine. A wafer 3 mm. wide was then cut from the inside of the buccal portion of the plaster models and the red plaster filling separated from the white plaster portion of the wafer.

Preparing the Photoelastic Model.—Catalin was the photoelastic material used in this study. Catalin is a good material for use in photoelastic studies because it can be worked and heated to a limited degree without developing residual stress patterns which appear in the polariscope. Catalin is a clear, colorless, phenol-formaldehyde resin and is optically sensitive. Outside the polariscope, catalin appears to be an ordinary plastic material.

The previously sanded surface of one of the red plaster filling models was placed on a piece of catalin, and a tracing of the plaster filling model was made on the catalin with a sharp instrument. The catalin model was roughed out with a band saw to within 2 mm. of the tracing. Then, with a rotor file, the model was filed to within less than 1 mm. of the tracing. Using a hand file and fine Swiss files, the exact outline of the catalin model was established. Three layers of masking tape were placed on the tooth-contacting surface of the catalin model to simulate the slight elasticity of dentin.

Each photoelastic model was tested immediately after it was made. This procedure was necessary because lines, similar to stress fringes, develop around the periphery of the catalin model shortly after the model is made. The development of lines around the periphery of the photoelastic material is called time edge effect. If more than one photoelastic model were made in advance of the testing procedure, time edge effect would occur in those models tested last.

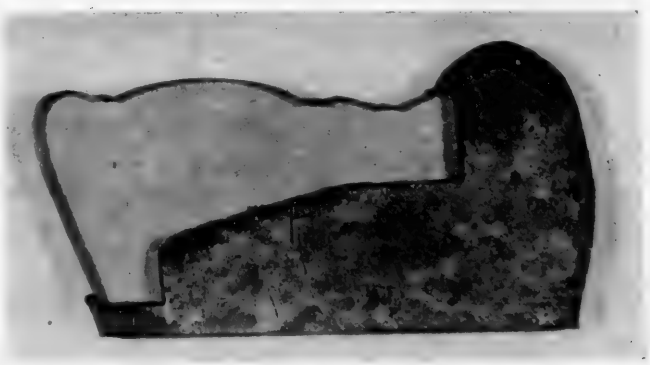

Fig. 5.—Catalin model in matrix ready for testing.

The Matrix for the Model.—A U-shaped holder, slightly larger than the mesial-distal length of the catalin model of the filling, was prepared from a similar piece of catalin. The catalin model was seated in the holder, the model and holder placed between two glass slabs, and the entire grouping held together in a vice. Wooden blocks were placed between the glass slabs and jaws of the vice to prevent the glass from breaking. S. S. White low-fusing metal (160° F.) was then poured into the mold formed by the glass slabs, the catalin model, and the holder. After the metal had solidified, its free surface was flattened and squared so that the metal could be seated firmly on the platform of the loading frame in the polariscope (Fig. 5).

Testing the Photoelastic Model.—The polariscope used in this study consisted of the following parts: (1) Light source: The light source consisted of a double band of green fluorescent lights in a plywood box. The box had a round opening twelve inches in diameter to emit the light. (2) Color filter: This filter was a transparent, green disk which covered the round opening in the plywood box. The disk filtered out all light outside a 600 Å band near 5,461 Å. (3) Polarizing filter: This filter screened out all light from the light source except light waves which vibrated vertically. (4) First quarter wave plate: This plate converted the vertical polarized light to circular polarized light so that the results of the test would be nondirectional in nature. (5) Loading frame and the mechanism for applying the load to the model: The platform was adjustable and could be arranged so that the photoelastic model could be in the middle of the field when viewed through the analyzer. The mechanism for applying the load was a lever arm on which a transverse cylindrical bar was mounted. The load was applied to the model at the point where the cylinder contacted the model. The lever arm was above the model, and the cylinder was on the bottom of the lever arm. The model and matrix could be adjusted from side to side on the platform so that the load could be applied at any point on the model. The lever arm was pivoted at one end. The load was applied to the free end of the lever arm by means of weights. (6) Second quarter wave plate: This plate reconverts the circular polarized light back to vertical polarized light. (7) Analyzer: This filter transmits only light vibrating in a horizontal plane.

In this study we used monochromatic light of 5,461 Å wave length (green). Monochromatic light was used instead of white light because it gives sharper and more definite stress patterns.

A copy camera with a Zeiss Apo-Tessar lens was used. The lens had a focal length of 45 cm. The diaphragm opening was f-16. Super Panchro-Press, type B, film was used. A one-second exposure time was used.

The matrix with the catalin model was placed on the platform of the loading frame in the polariscope and was loaded first on the mesial pit of the model. The picture was taken with this load (163.5 pounds), which was then removed as quickly as possible, and the residual stresses were allowed to creep out of the model. The load was again applied in the same manner to the crest of the triangular ridge just distal to the mesial pit. Another picture was taken.

When the catalin models were subjected to stress, the fringe orders were counted at three different points: (1) on the pulpal wall just distal to the pulpal-axial line angle, Point A, (2) at the pulpal-axial line angle, Point B, and (3) on the axial wall slightly to the gingival of the pulpal-axial line angle, Point C. From the beginning of one bright line to the beginning of the next bright line in the photographs is termed a "fringe." The fringe order at a point is the number of the fringe at the point counting from a point at which the stress is zero. The fringe order is directly proportional to shear stress in the model.

After the photographs were made, the fringe orders were also counted on the photographs. This procedure was followed in order to check ourselves on the count made when the models were in the polariscope.

DISCUSSION

Since this investigation was a qualitative study, the numbers in Tables I and II are valid only when compared with numbers in these tables or another study exactly like this one. The numbers mean nothing so far as quantitative measurements are concerned. It should be remembered that we are trying to determine which, if any, of the principles tested would aid in reducing the stresses in a restoration placed in a Class II cavity preparation. It should also be pointed out that in this study we were working with materials which are unlike those found in human teeth (dentin and enamel) in that they are homogeneous, isotropic, and elastic.

TABLE I

LOAD APPLIED ON MESIAL PIT

MODEL NO.	PULPAL WALL DEATH	AXIAL WALL	PULPAL WALL	PULPAL-AXIAL LINE ANGLE	NO. STRESS LINES		
					A	B	C
1	sh	v	h	sq	9	4	3
2	d	v	h	sq	6	4	4
3	sh	v	h	r	6	7	6
4	d	v	h	r	5	3	5
5	sh	v	sl	sq	4	5	6
6	d	v	sl	sq	3	3	4
7	sh	v	sl	r	4	1	6
8	d	v	sl	r	3	5	7
9	sh	sl	h	sq	5	5	1
10	d	sl	h	sq	2	1	0
11	sh	sl	h	r	4	1*	1
12	d	sl	h	r	0	0	1
13	sh	sl	sl	sq	1	1	2
14	d	sl	sl	sq	1	3	4
15	sh	sl	sl	r	1	3	6
16	d	sl	sl	r	1	3	6

Key:
sh—shallow (10 mm.).
d—deep (15 mm.).
v—vertical.
h—horizontal.
sl—sloping.
sq—square.
r—rounding.
*May be in error.

Shallow Versus Deep Occlusal Steps.—In general, the cavity preparations with the shallow occlusal step permitted greater stress to develop than did those cavity preparations with the deep occlusal step (Fig. 6). The difference between the shallow and deep occlusal step preparations was particularly noticeable when the load was applied on the transverse ridge of the occlusal surface.

A study of Tables I and II reveals that, regardless of occlusal depth, when the load was applied in the mesial pit of the occlusal surface, a lower fringe order developed at Points A, B, and C than when the load was applied on the transverse ridge of the occlusal surface. The photographs also showed that

when the load was applied in the mesial pit more stress was thrown onto the gingival wall, with a higher fringe order developing in the gingival region of the restoration.

It is apparent that greater depth of filling material allows for a better distribution of stress in the restoration. The influence of bulk of filling material and point of load application will also be noted rather pointedly when other principles are discussed.

TABLE II

LOAD APPLIED ON TRANSVERSE RIDGE

MODEL NO.	PULPAL WALL DEATH	AXIAL WALL	PULPAL WALL	PULPAL-AXIAL LINE ANGLE	NO. STRESS LINES		
					A	B	C
1	sh	v	h	sq	10	5	3*
2	d	v	h	sq	7	7	3
3	sh	v	h	r	11	6	6
4	d	v	h	r	7	5	3
5	sh	v	sl	sq	3	5	6
6	d	v	sl	sq	1	3	4
7	sh	v	sl	r	1	4	6
8	d	v	sl	r	2	4	5
9	sh	sl	h	sq	18	11	1
10	d	sl	h	sq	8	6	0
11	sh	sl	h	r	14	12	2
12	d	sl	h	r	6	6	1
13	sh	sl	sl	sq	6	3	2
14	d	sl	sl	sq	1	3	4
15	sh	sl	sl	r	6	2	8
16	d	sl	sl	r	1	4	7

Key:
sh—shallow (10 mm.).
d—deep (15 mm.).
v—vertical.
h—horizontal.
sl—sloping.
sq—square.
r—rounding.
*May be in error.

Rounding and Sloping Axial Walls Versus Vertical Axial Walls.—It appears from Table I that rounding and sloping the axial wall are beneficial because there is a great reduction in the fringe order in the test models. Among the factors which were responsible for this reduction of stress were the point of load application and the position of the pulpal-axial line angle. Sloping and rounding the axial wall of the cavity preparation placed the pulpal-axial line angle farther away from the load point (mesial pit), and there was nothing to interfere with the distribution of stress between the load point and the gingival wall of the cavity preparation.

From Table II, it can be seen that rounding and sloping the axial wall was much less beneficial. In these specimens higher stress was induced in the pulpal-axial line angle area (Points A and B) because sloping the axial wall placed the pulpal-axial line angle directly beneath the point of load application. Whenever a load is placed directly over an angle, the stress tends to be concentrated at the angle.

It should be mentioned that rounding and sloping the axial wall of the cavity preparation could be a hazardous undertaking and an undesirable one from the standpoint of pulpal exposure.

Rounding and Sloping Versus Horizontal Pulpal Walls.—Rounding and sloping the pulpal wall consistently produced the best general distribution of stress. As can be seen from Tables I and II, the amount of stress was reduced in almost every case where the rounding and sloping pulpal wall was used, instead of leaving the pulpal wall flat.

A. *B.*

Fig. 6.—Photographs of catalin models showing fringe patterns which developed with occlusal step depths of 10 mm. (*A*) and 15 mm. (*B*). In both models the pulpal and axial walls of the cavity preparation were flat, the pulpal-axial line angle was square, and the load was applied in the mesial pit.

A. *B.*

Fig. 7.—Photographs of the same catalin model showing fringe patterns which developed when load was applied in mesial pit (*A*) and on transverse ridge (*B*). In both models the occlusal step was deep (15 mm.), the pulpal wall was rounded and sloping, the axial wall horizontal, and the pulpal-axial line angle rounded.

It will be noted in Table II that there was a dramatic reduction in stress when the pulpal wall was sloped and rounded, as compared with leaving it horizontal (Fig. 7). It is our opinion that this marked reduction in stress is due to the fact that rounding and sloping the pulpal wall of the Class II cavity preparation provide for a greater thickness of filling material and a better distribution of stress at a point where it is needed.

Round and Square Pulpal-Axial Line Angles.—In some cases where the pulpal-axial line angle was rounded there was a reduction of stress (Tables I and II). However, the amount of stress reduction is not significant. The difference in the fringe orders is so small and inconsistent that there can be no conclusion drawn except that just rounding the pulpal-axial line angle made very little difference in so far as stress magnitude is concerned.

SUMMARY AND CONCLUSIONS

1. A method is suggested for studying the stress patterns which are induced in the restoration as the result of different Class II cavity preparations.

2. Mesiodistal cross sections of Class II cavity preparations with flat pulpal walls, flat axial walls, rounded and sloping pulpal walls, rounded and sloping axial walls, square pulpal-axial line angles, round pulpal-axial line angles, shallow occlusal steps (10 mm.), and deep occlusal steps (15 mm.) were studied in various combinations. Each principle was tested eight different times.

3. There was less concentration of stress and thus a better distribution of stress in those restorations placed in cavity preparations which allowed for a greater depth of the filling material (deep occlusal steps and rounded and sloping pulpal walls).

4. Rounding and sloping the axial wall did not consistently reduce stress in the restoration. Because of the possibility of pulpal involvement, this procedure is not recommended for Class II cavity preparations.

5. The point of load application has a definite bearing on the stress patterns induced in the restoration. The farther away from the pulpal, axial, and gingival cavity walls the load is applied, the less will be the stress around these cavity walls.

6. Slight rounding of the pulpal-axial line angle had little effect in reducing stress in the filling regardless of the point of load application. These results support previous findings that rounding the pulpal-axial line angle had little effect in increasing the resistance of the restoration to fracture. Nevertheless, rounding the pulpal-axial line angle is recommended because this procedure provides for a slight increase in the thickness of the filling material, and because there is more concentration of stress around an acute angle.

7. There was a marked reduction of stress when the pulpal wall was rounded and sloped. These findings also support the results of the investigation referred to previously in which it was shown that when the pulpal wall of the Class II cavity was rounded and sloped, the resistance of the silver amalgam restoration to fracture was markedly increased. Therefore, rounding and sloping the pulpal wall of the Class II cavity preparation are recommended as a most desirable procedure.

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TIME STUDIES OF THE PENETRATION OF EXTRACTED HUMAN TEETH BY RADIOACTIVE NICOTINAMIDE, UREA, THIOUREA, AND ACETAMIDE

I. Diffuse Penetration From the Enamel Surface

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THE availability of radioisotope-labeled compounds has greatly extended the possibility of studying the permeability of dental structures (Bartelstone 1950, Leicester 1949). The finding of a rapid diffuse penetration of intact enamel by C^{14}-labeled urea (Wainwright and Lemoine 1950) has suggested further studies of the time of penetration of C^{14}-urea and other labeled compounds.

Freshly extracted teeth and, in some cases, formol-fixed teeth were superficially dried with absorbent paper and air blast and embedded directly in Selectron (Wainwright 1949B). The surface to be treated was exposed with a round bur and the tooth immersed in distilled water until used. Solutions of the isotope-labeled compounds were prepared so that the activity was approximately 1 mc. per milliliter.† Approximately 0.02 ml. of labeled solution was placed on the exposed surface, and the tooth was placed in a humid atmosphere at room temperature. The teeth treated with C^{14}-acetamide were placed in a humid atmosphere which was maintained at 37° C., as suggested by Jarabak (1941).

Upon removal from the humidifier, part of the excess solution was recovered by washing the surface with a stream of approximately 15 ml. distilled water within thirty seconds. The teeth were sectioned and surfaced under oil (Wainwright and Lemoine 1950). The radioactivity of the treated specimens was about 2,000 to 3,000 CPM. Survey radioautographs (Wainwright 1949A) were made with a constant exposure of four days.

The labeled compounds were synthesized by A. R. Ronzio, A. Murray, III, and C. W. Bills (1947, 1949, and 1950), and supplied by the Bio-Medical Research Group of the Los Alamos Scientific Laboratory. The compounds were synthesized with carbon^{14} and sulfur^{35} obtained on allocation from the Isotope Division of the Unites States Atomic Energy Commission.

C^{14}-Nicotinamide.—Labeled nicotinamide offers the possibility of testing the penetration of an organic substance which can occur normally in the mouth. Table I gives the time of exposure to the solution and the extent of diffuse

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†The specific activities were: nicotinamide, 35 μc per milligram; urea, 55.6 μc per milligram; thiourea, 4 μc per milligram; acetamide, 50 μc per milligram.

TABLE I

DIFFUSE PENETRATION OF ENAMEL BY CARBON^{14}-LABELED NICOTINAMIDE

TOOTH		AGE OF PATIENT	REASON FOR LOSS	SPECIMEN			RADIO-AUTO-GRAPH NO.	PENETRATION					
								TIME		ENAMEL		DENTIN	
NO.	LOCATION	(YR.)		NO.	SURFACE TREATED	LEVEL OF TRANSVERSE SECTION		IN HUMIDIFIER	TOTAL*	MAXIMUM DEPTH (MM.)	PROPORTION OF ENAMEL THICKNESS (%)	MAXIMUM DEPTH (MM.)	PROPORTION OF DENTIN THICKNESS (%)
392†	UL5	31	Caries	1071	Lingual	Middle	A826	0	12 min.	–	–	–	–
				1072		Cervical	A827		13 min.	–‖			
396‡	UR8	30§	Malposed	1073	Buccal	Middle	A830	30 min.	43 min.	–‖ ¶	–	–	–
				1074		Cervical	A831		47 min.	–¶	–	–	–
397#	UR6	30§	Pulpitis	1075	Buccal	Middle	A829	60 min.	76 min.				
				1076		Cervical	A828		79 min.	0.4	100	–	–
398**	UR8	24	Pericoronitis	1077	Buccal	Middle	A839	2 days	2 days	0.1	1	–	–
				1078		Cervical	A840			–††	–	–	–
399‡‡	UR1	20	Paradentosis	1079	Labial	Middle	B178	3 days	3 days	0.6§§	100	1.1	6
				1080		Cervical	B179			0.6§§	100	1.4	70

*Total time = time in humidifier + time of sectioning, xylene wash, and drying by air blast.

†Extracted two days before experiment; kept in moist gauze one day. Embedded in Selectron one day before experiment. Mesio-occlusal amalgam filling with cement base; dentin silver nitrated.

‡Formol fixed 196 days. Embedded in Selectron one day before experiment. Carious occlusal fissures.

§Patient D. D.

‖Surface caries penetrated.

¶Enamel lamellae penetrated.

#Formol fixed 196 days. Embedded in Selectron one day before experiment. Occlusal amalgam filling, dentin silver nitrated. Disto-occlusal caries into pulp.

**Formol fixed 164 days. Embedded in Selectron one day before experiment.

††Lamellar penetration into dentin.

‡‡Formol fixed eighty-two days. Embedded in Selectron one day before experiment.

§§Weak diffuse penetration of all dentin.

penetration as measured on the radioautographs. Ten specimens are described from one fresh tooth and four formol-fixed teeth. Only lamellae or defects in the enamel were penetrated until the seventy-nine-minute specimen. In this specimen penetration was detected through the entire enamel, while in the three-day specimen, penetration proceeded 1.4 mm. into the dentin as well.

Typical radioautographs and the respective specimens are pictured in Fig. 1. The first radioautograph shows a faint surface uptake. Greater surface uptake and penetration of carious enamel are seen in the thirty- and sixty-minute radioautographs, while the three-day radioautograph shows diffuse and deep penetration.

C^{14}-Urea.—The distances penetrated by radioactive urea are given in Table II for sixteen specimens from four formol-fixed teeth. The twenty-minute specimens showed diffuse penetration through 0.5 mm. of enamel. The distance increased with time until by one day, 1.3 mm. of enamel and 3.1 mm. of dentin were penetrated.

Typical radioautographs are shown in Fig. 2 for specimens exposed to the solution for periods of thirty-nine minutes to two days. The progress of the urea is readily followed in the successive radioautographs. In the two-day radioautograph, the radioactive urea streaks toward the dentin apparently along the bands of Schreger. Exposed cervical dentin is seen to be penetrated in the one- and two-day radioautographs. The latter shows a dense band of penetration to the pulp chamber.

S^{35}-Thiourea.—A compound closely related chemically to urea with the same extreme solubility, and offering the advantage of being labeled by a different isotope is sulfur^{35}-labeled thiourea. Although thiourea cannot be used for oral therapy as it is a readily absorbed thyrotoxin, for in vitro experiments it is very useful for tests of enamel permeability. Nine unfixed teeth were treated with this compound. The extent of penetration in the seventeen specimens is described in Table III. Slight uptake on the enamel surface was noted in the radioautographs of the six- and seven-minute specimens. In all other specimens, with times of exposure to the solution of thirty-four minutes to four days, there was penetration at some point to the dento-enamel junction or beyond. In the thirty-four-minute samples this occurred through 1.1 mm. of enamel.

The representative radioautographs of Fig. 3 illustrate the remarkable penetrating properties of this compound. The first shows a surface uptake within seven minutes, while the thirty-four-minute radioautograph and all the others show a broad diffuse penetration of enamel. In the sixty-minute and one-day radioautographs there are dark streaks through the enamel which suggest great concentrations in lamellae.

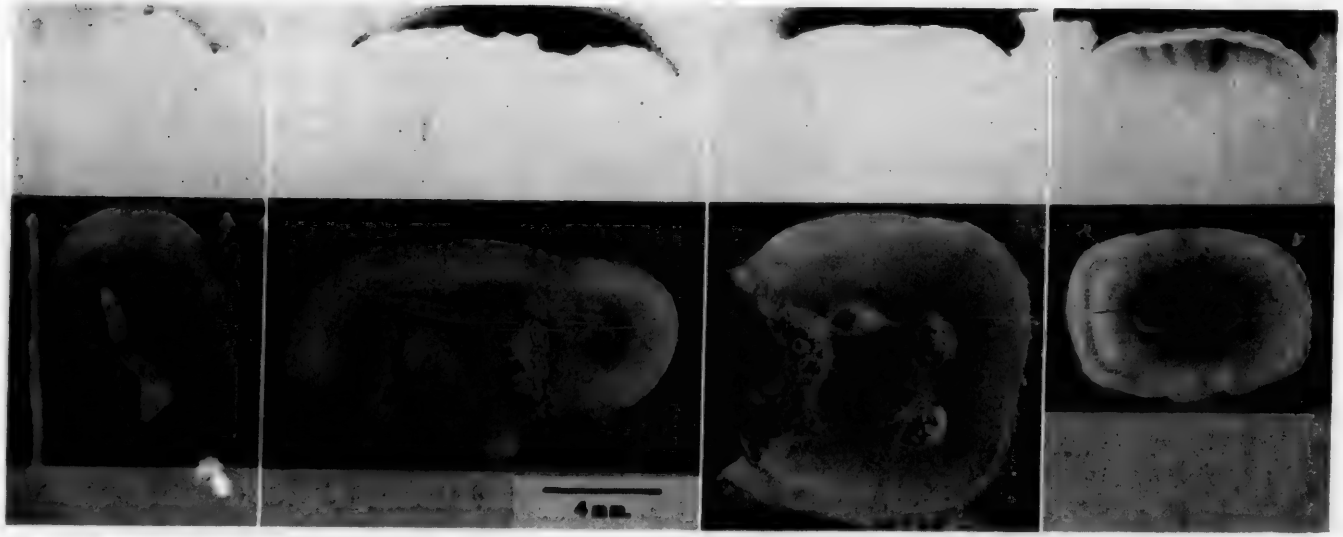
C^{14}-Acetamide.—A product of protein metabolism closely resembling urea is acetamide. Its chemical structure is related to urea, and it is highly soluble. The synthesized compound was labeled with C^{14} in the carbamide group. The distances penetrated, at 37° C., in the enamel of ten specimens from five freshly

TABLE II

DIFFUSE PENETRATION OF ENAMEL BY CARBON^{14}-LABELED UREA

TOOTH* NO.	TOOTH* LOCATION	AGE OF PATIENT (YR.)	REASON FOR LOSS	SPECIMEN NO.	SPECIMEN SURFACE TREATED	SPECIMEN SECTIONED LONGITUDINALLY THROUGH CUSP:	RADIOAUTOGRAPH NO.	PENETRATION TIME IN HUMIDIFIER	PENETRATION TIME TOTAL†	ENAMEL MAXIMUM DEPTH (MM.)	ENAMEL PROPORTION OF ENAMEL THICKNESS (%)	DENTIN MAXIMUM DEPTH (MM.)	DENTIN PROPORTION OF DENTIN THICKNESS (%)
342‡	LR8	30	Impacted (unerupted)	977	Lingual	Distal	A734	0	13 min.	–§	–	–	–
				978		Mesial	A725			–§	–	–	–
				979	Buccal	Mesial	A726	10 min.	20 min.	0.5	50	–	–
				980		Distal	A727			0.5	29	–	–
344‖	LR8	21	Pericoronitis	985	Buccal	Mesial	A730	30 min.	39 min.	0.7	100	0.1	4
				986		Distal	A731			0.2	18	–	–
				987	Lingual	Mesial	A746	2 days	2 days	0.3	43	–	–
				988		Distal	A745			0.5	62	–	–
343¶	LR8	29	Pericoronitis	983	Buccal	Mesial	A728	1 day	1 day	0.5	100	–	–
				984		Distal	A729			1.3	100	3.1	100
				981	Lingual	Distal	A737	2 days	2 days	1.3	100	1.0	37
				982		Mesial	A735			1.3	100	1.1	33
345#	UL8	20	Malposed	989	Buccal	Mesial	A732	60 min.	67 min.	0.6	50	–	–
				990		Distal	A733	60 min.	69 min.	0.4	36	–	–
				991	Lingual	Mesial	A750	3 days	3 days	0.3	43	–	–
				992		Distal	A749	3 days	3 days	0.7	100	1.6	62

*Embedded in Selectron two days before experiment. Stored one day in distilled water at 5° C.
†Total time = time in humidifier + time of sectioning, xylene wash, and drying by air blast.
‡Formol fixed 150 days.
§No detectable surface uptake.
‖Formol fixed 139 days.
¶Formol fixed 105 days.
#Formol fixed 174 days.



Time in Humidifier: 0 min. | 30 min. | 60 min. | 3 days

Fig. 1.—Penetration of enamel by carbon^{14}-labeled nicotinamide occurs only through carious areas and lamellae in the 0-, 30-, and 60-minute specimens. Diffuse penetration through intact enamel into the dentin is observed in the 3-day specimen. The time in the humidifier does not include the time used in sectioning. The total time for each specimen is given in Table I.

(0 min.: Spec. 1071, Neg. 15133; R.A.—A826, Neg. 15169.
30 min.: Spec. 1073, Neg. 15124; R.A.—A830, Neg. 15160.
60 min.: Spec. 1075, Neg. 15127; R.A.—A820, Neg. 15175.
3 days: Spec. 1079, Neg. 15260; R.A.—A841, Neg. 15256.)

TABLE III

DIFFUSE PENETRATION OF ENAMEL BY SULFUR^{35}-LABELED THIOUREA

TOOTH		AGE OF PATIENT	REASON FOR LOSS	SPECIMEN			RADIO-AUTO-GRAPH NO.	PENETRATION					
								TIME		ENAMEL		DENTIN	
NO.	LOCATION	(YR.)		NO.	SURFACE TREATED	LEVEL OF TRANSVERSE SECTION		IN HUMIDIFIER	TOTAL*	MAXIMUM DEPTH (MM.)	PROPORTION OF ENAMEL THICKNESS (%)	MAXIMUM DEPTH (MM.)	PROPORTION OF DENTIN THICKNESS (%)
407	LR2	60‡	Paradentosis	1111	Mesial	Middle	A864	0	6 min.	–†	–	–	–
				1112		Cervical	A874		7 min.	–†	–	–	–
408	LR3			1113	Distal	Middle	A875	30 min.	34 min.	1.1	100	–	–
				1114		Cervical	A865		36 min.	0.3	100	0.1	5
409	UL1			1115	Labial	Middle	A876	60 min.	68 min.	0.6	100	0.5	20
				1116		Cervical	A877		70 min.	0.4	100	0.2	9
410	UL3			1117	Mesial	Middle	A879	60 min.	68 min.	0.6	100	0.1	5
				1118		Cervical	A878		69 min.	0.5	100	0.1	4
411	UL5			1119	Distal	Middle	A880	1 day	1 day	0.6	100	0.4	14
				1120		Cervical	A881			0.3	100	0.2	10
412	LL1			1121	Mesial	Middle	A882	1 day	1 day	0.4	100	0.3	21
				1122		Cervical	A869			0.3	100	0.3	14
413	LL2			1123	Distal	Middle	A884	4 days	4 days	0.4	100	1.2	100
				1124		Cervical	A888			0.4	100	0.4	25
414§	LR8	27	Impacted (partly erupted)	1126	Buccal	Cervical	A886	4 days	4 days	0.5	100	4.0	100
415§	UL8	28	Caries‖	1127	Mesial	Middle	A887	4 days	4 days	0.9	100	0.7	64
				1128		Cervical	A885			0.4	100	1.3	71

*Total time = time in humidifier + time of sectioning, xylene wash, and drying by air blast.

†Slight surface uptake.

‡Patient L. G. Teeth extracted and embedded in Selectron three days before experiment. Stored in distilled water two days at 5° C.

§Extracted four days before experiment. Stored one day in moist gauze. Embedded in Selectron three days before experiment. Stored in distilled water two days at 5° C.

‖Caries into dentin of distal half of occlusal and lingual surfaces.

extracted teeth are listed in Table IV. Within seven minutes an uptake was noted on the surface of the enamel. After thirty-four minutes, penetration had occurred through the dentoenamel junction and 1.4 mm. into the dentin. This rapid diffuse penetration was found in greater degree in the sixty-minute and three-day specimens.

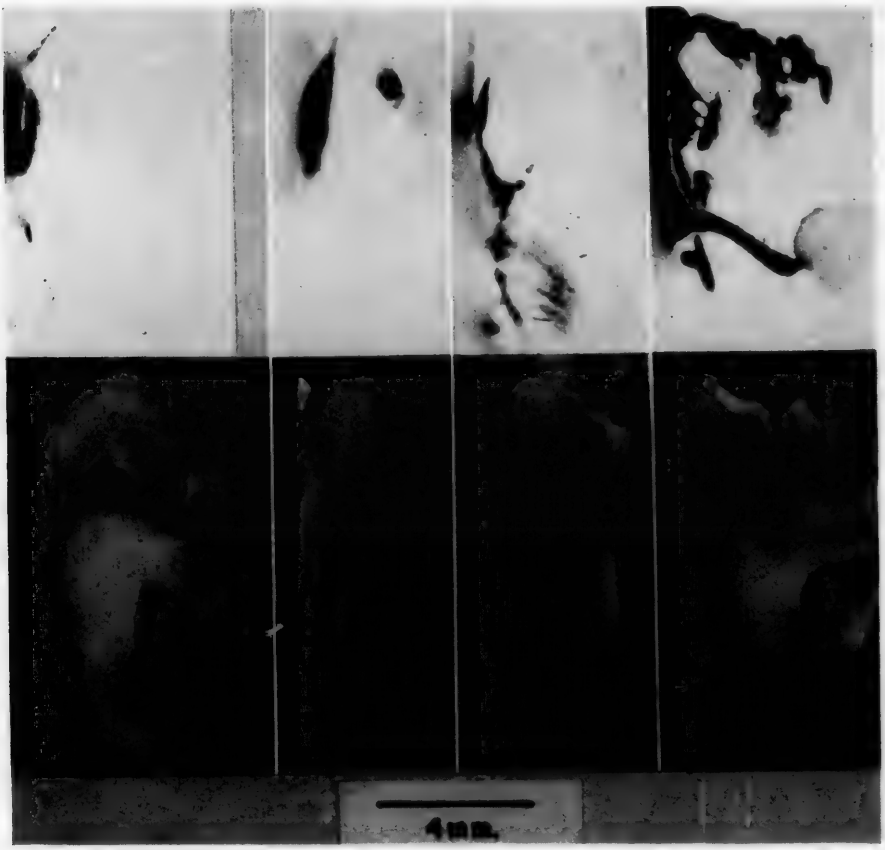


Fig. 2.—Diffuse penetration of intact enamel by carbon^{14}-labeled urea is rapid. The dentoenamel junction was reached (0.7 mm.) in 39 minutes in the first specimen. In the 2-day specimen the urea penetrated to the pulp through the exposed cervical dentin. The time in the humidifier does not include the time used in sectioning. The total time for each specimen is given in Table II.

(30 min.: Spec. 985, Neg. 15128; R.A.—A730, Neg. 15199.
60 min.: Spec. 989, Neg. 15115; R.A.—A732, Neg. 15198.
1 day: Spec. 984, Neg. 15130; R.A.—A729, Neg. 15200.
2 days: Spec. 982, Neg. 15131; R.A.—A735, Neg. 15201.)

Fig. 4 shows typical radioautographs with times of penetration of thirty-four minutes, seventy minutes, and three days. All the radioautographs show a deep diffuse penetration through the enamel into the dentin. The areas penetrated are broader in the longer periods of exposure to the C^{14} acetamide.

TABLE IV

DIFFUSE PENETRATION OF ENAMEL BY CARBON^{14}-LABELED ACETAMIDE

TOOTH*		AGE OF PATIENT (YR.)	REASON FOR LOSS	SPECIMEN			RADIO-AUTO-GRAPH NO.	PENETRATION					
								TIME		ENAMEL		DENTIN	
NO.	LOCATION			NO.	SURFACE TREATED	LEVEL OF TRANS-VERSE SECTION		IN HU-MIDIFIER	TOTAL†	MAXI-MUM DEPTH (MM.)	PROPORTION OF ENAMEL THICKNESS (%)	MAXI-MUM DEPTH (MM.)	PROPORTION OF DENTIN THICKNESS (%)
451‡	UR4	37§	Paraden-tosis	1287	Buccal	Middle	B156	0	7 min.	–‖	–	–	–
				1288		Cervical	B167		8 min.	–‖	–	–	–
458¶	UR5	29	Paraden-tosis	1289	Buccal	Middle	B168	30 min.	35 min.	0.3	75	–	–#
				1290		Cervical	B157		34 min.	0.3	100	1.4	70#
456	LL8	25	Impacted (unerupt-ed)	1291	Buccal	Middle	B159	60 min.	69 min.	0.9	100	–	–**
				1292		Cervical	B160		70 min.	0.6	100	0.6	–††
455‡‡	UR8	37§	Paraden-tosis	1293	Distal	Middle	B169	3 days	3 days	1.1	100	3.4	100**
				1294		Cervical	B170			0.5	100	2.8	100**
453§§	UR6	37§	Paraden-tosis	1297	Buccal	Middle	B171	3 days	3 days	1.1	100	3.1	100** ‖‖
				1298		Cervical	B172			0.4	100	3.1	100** ‖‖

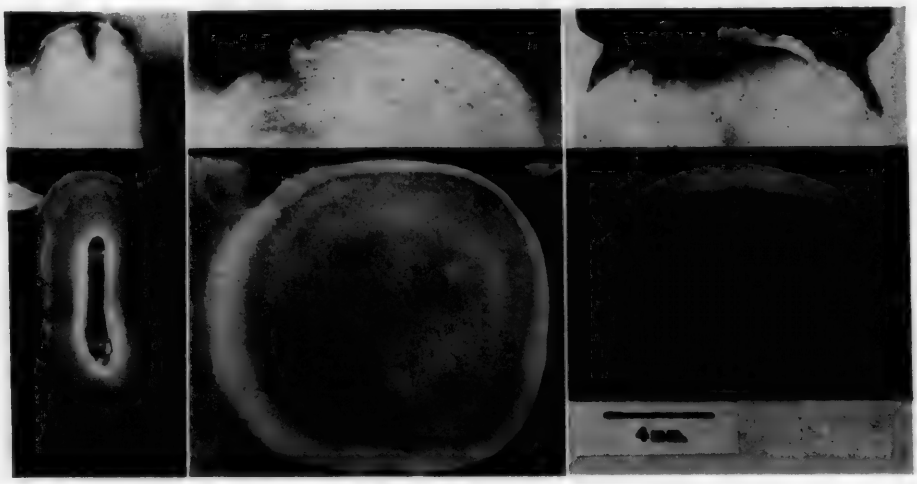
*Extracted and embedded in Selectron one day before experiment.
†Total time = time in humidifier + time of sectioning, xylene wash, and drying by air blast.
‡Distal caries into dentin.
§Patient O. C.
‖Very slight surface uptake.
¶Mesial-occlusal-distal amalgam filling. Dentin silver nitrated.
#Weak diffuse penetration of all dentin.
** Diffuse penetration of all dentin.
††Weak diffuse penetration of most dentin. Pulp not in plane of section.
‡‡Fissures stained brown.
§§Mesio-occlusal caries into pulp.
‖‖Carious dentin and zinc oxide and eugenol filling penetrated.

Fig. 3.—Diffuse penetration of intact enamel by sulfur^{35}-labeled thiourea. The first specimen shows no penetration in 7 minutes. The 34-minute specimen shows penetration up to the dentoenamel junction, while subsequent specimens show penetration of the dentin as well. The dark line through the enamel of the 1-day radioautograph is considered to be penetration through a lamella. The time in the humidifier does not include the time used in sectioning. The total time for each specimen is given in Table III.

(0 min.: Spec. 1112, Neg. 15118; R.A.—A874, Neg. 15172.
30 min.: Spec. 1113, Neg. 15119; R.A.—A875, Neg. 15162.
60 min.: Spec. 1116, Neg. 15116; R.A.—A877, Neg. 15161.
1 day: Spec. 1119, Neg. 15117; R.A.—A880, Neg. 15196.
4 days: Spec. 1124, Neg. 15134; R.A.—A888, Neg. 15163.)

CHROMATOGRAPHIC IDENTIFICATION OF THE UREA MOLECULE AFTER PENETRATION

As pointed out by Manley (1950), the possibility exists that the urea molecule is split on the surface of the enamel, and the substance penetrating could be carbonate ion. The radioautograph records only the radiations from the C^{14} atom and does not detect the urea molecule as such. To identify the penetrated material, dentin was cut from two teeth which had been treated on the enamel surface with C^{14}-urea. Water extracts of the dentin were placed on chromatogram strips and control strips run with the original C^{14}-urea solution. The results, seen in Fig. 5, are identical for each of the four strips, indicating that it is the urea molecule which penetrated into the dentin. Carbonate would have traveled a very much shorter distance on the strip.



Time in Humidifier 30 min. 60 min. 3 days

Fig. 4.—Diffuse penetration of intact enamel by carbon^{14}-labeled acetamide proceeds rapidly. In the 34-minute specimen the acetamide has penetrated the enamel and two-thirds of the thickness of dentin. The time in the humidifier does not include the time used in sectioning. The total time for each specimen is given in Table IV.

(30 min.: Spec. 1290, Neg. 15257; R.A.—B157, Neg. 15177.
60 min.: Spec. 1292, Neg. 15269; R.A.—B160, Neg. 15173.
3 days: Spec. 1293, Neg. 15262; R.A.—B169, Neg. 15255.)

DISCUSSION

Confirmation of the rapid diffuse penetration of grossly intact enamel by urea and the finding of this property in nicotinamide, thiourea (a toxic substance not suitable for oral use), and acetamide make possible a new concept of the types of posteruptive changes in the intact tooth. If, to this information, there can be added in vivo observations and studies with labeled proteins and other large molecules normally concerned in tooth metabolism, a more definite understanding may be achieved of the physiologic as well as physical and chemical processes occurring in enamel.

The finding that a large number of substances (Wainwright 1951) penetrate minute enamel defects, such as caries, cracks, and lamellae, and exposed dentin, and yet do not show the property of diffuse penetration, emphasizes the concept of enamel vulnerability. The latter studies showed a great incidence of defects and emphasized the fact that teeth have many open pathways for the entrance of damaging agents. Thus the picture unfolds of several levels of physical, chemical, and physiologic change in enamel, dentin, and cementum. These levels are determined by the properties of the dental tissues themselves as well as the electrical, chemical, and physical properties of the substances in the environment of the tooth.

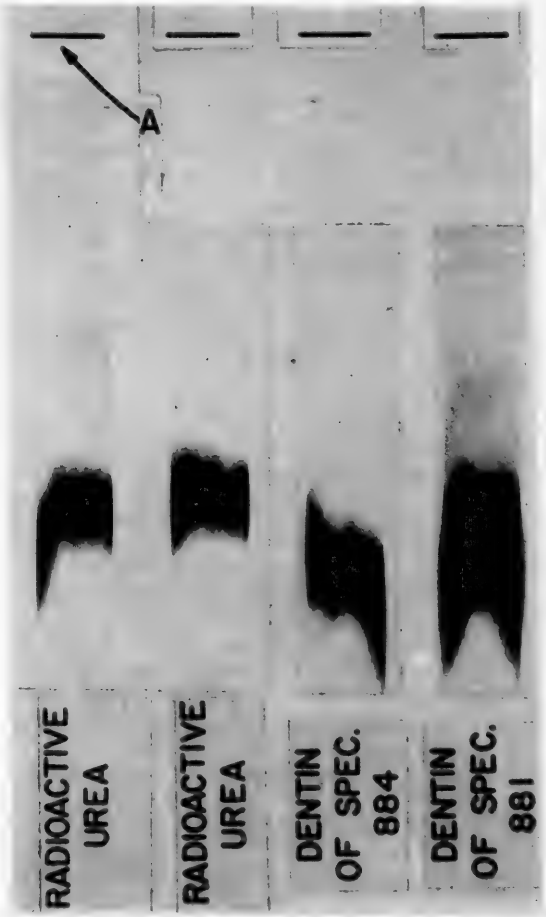


Fig. 5.—Chromatographic identification of the urea molecule in dentin extracts after enamel penetration.

Recent histologic investigations of enamel lamellae have presented evidence for their contents being of cellular origin (Gottlieb 1947); "pseudolamellae" are described as being the pathway of deep penetration of caries

(Frisbie, Nuckolls, and Saunders 1944); or as arising from traumatic cracks in the enamel (Sognnaes 1950). The difficulty of interpreting evidence from ground sections alone is cited by Bodecker and Lefkowitz (1937) and Berggren (1947). These authors also noted the prominent role of the bands of Schreger in distributing dyes in the enamel. Clarification of the nature and functions of these structures as well as the pathways of diffuse penetration may also solve the problems of enamel physiology and pathology.

SUMMARY

1. Rapid diffuse penetration of grossly intact enamel by isotope-labeled solutions of nicotinamide, urea, thiourea, and acetamide was determined radioautographically in twenty-three extracted human teeth.

2. Eight of the teeth were formol fixed. No effect of fixation, age, sex, or tooth location was observed.

3. Nicotinamide penetration was the slowest. The earliest diffuse penetration observed was through 0.4 mm. of enamel within seventy-nine minutes.

4. Urea, thiourea, and acetamide penetrated more rapidly. Urea penetrated one-half the thickness of the enamel (0.5 mm.) within twenty minutes. Thiourea (a toxic substance not suitable for oral use) and acetamide penetrated the entire thickness of the enamel (1.1 and 0.3 mm., respectively) within thirty-four minutes.

5. After a slightly longer period, all four compounds penetrated the dentoenamel junction at intervals and went into the dentin. In a few teeth the pulp was reached.

6. Chromatographic examination of dentin extracts from two teeth, to the enamel surfaces of which C^{14}-urea had been applied, identified the compound which penetrated as the urea molecule.

The suggestions of Dr. Wright Langham, leader, Bio-Medical Research Group, Dr. Thomas L. Shipman, leader, Health Division, and Dr. John H. Manley, technical associate director, Los Alamos Scientific Laboratory, have been essential to the conduct of the investigation. Dr. Howard Belgorod, Dental Clinic, Los Alamos Medical Center, has assisted generously and inspired the author in all phases of the study. Grateful acknowledgment is made to Dr. Anthony R. Ronzio, Arthur Murray, III, and C. W. Bills, who prepared the isotope-labeled compounds, and to Miss Agnes Williams who prepared the radioautographs. Teeth were supplied through the cooperation of the Dental Staff of the Los Alamos Medical Center: Drs. Melvin Morrow, director, Nathan Peretzman, Howard Belgorod, F. A. Lemoine, I. R. Quijada, Harold Barrie, and Charles Paraskis.

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CHANGES IN THE TEETH AND JAWS OF NEONATAL WEBSTER MICE AFTER ADMINISTRATION OF NaF AND CaF_2 TO THE FEMALE PARENT DURING GESTATION

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INTRODUCTION

SEVERAL studies have shown that certain concentrations of fluoride appear capable of passing through placental membranes.[3, 7, 10] In view of this fact, results of fluoride administration during pregnancy, as reflected in the teeth or adjacent structures of the newborn child or animal, should be of interest. It is the purpose of this paper to report findings as seen in certain teeth of neonatal Webster mice following administration of NaF and CaF_2, in selected dilutions, to the female parents.

Reports in the literature on the specific effects of fluoride administered during the gestation period of the female parent are lacking. Studies such as those of Hicks[5] on the administration of metabolic inhibitors during gestation have usually been concerned with structures other than the teeth. The recent work of Kreshover and Clough[8] approaches the problem of prenatal influences on tooth development by inducing maternal pyrexia.

MATERIALS AND METHODS

In order to have a large number of dated, pregnant mice available for these experiments, fifteen breeding cages and jars were organized with young adult Webster mice in rooms at a constant temperature of 75° F. One male was allotted to every three or four females. Upon daily examination, females with vaginal plugs were placed in other quarters, and new young adult females were added to the breeding units. In instances where there seemed to be an absence of pregnancies, males and females were exchanged for new animals. In this manner a study was conducted on 245 pregnancies in which the animals received fluorides (eighty mice receiving NaF via drinking water, fifty mice receiving CaF_2 via drinking water, sixty mice receiving NaF via injection, fifty-five mice receiving CaF_2 via injection) and on twenty-five additional pregnancies that served as controls. Many of the newborn animals were devoured at birth, thus making an accurate count of numbers of mice born impractical.

Administration of fluorides was usually started between the fifth and the tenth days of pregnancy, but in some instances was not given until the fifteenth day, and on still other occasions, was started immediately after observation of the vaginal plugs. Newborn mice were obtained immediately after birth and

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frequently at birth. One or two animals from each litter were sacrificed, and the heads fixed in 10 per cent formalin, decalcified, and sectioned. Imbedded heads were cut at 6 μ from the snout tip at right angles to the inferior border of the mandible. Sections were stained with hematoxylin and eosin. Mandibular incisors were selected for the illustrations because sections from this area were always more uniform. The effects on the other teeth, however, were also observed.

Reagent grades of NaF and CaF_2 were diluted 1:1,000 (1,000 ppm) and 1:50,000 (20 ppm), and were used for intraperitoneal injections and drinking water, respectively. When injections were given, 0.10 c.c. of the 1:1,000 solution was administered daily. This was the equivalent of 100 μg of NaF or CaF_2 per injection. Via drinking water, each pregnant Webster mouse consumed 6 to 8 c.c. of solution daily, and sometimes more. It is estimated that approximately one-half of this amount may have been lost by waste and evaporation, so that each mouse actually consumed 3 to 4 c.c. daily. In these cases, consumed fluorides ranged from 60 to 80+ μg daily. Fluorides were stored in polyethylene flasks at 10° C. Because CaF_2 was not completely soluble in the 1:1,000 dilution, it was necessary to shake the container vigorously each time before use.

RESULTS

The effect of NaF and CaF_2 on developing teeth in the newborn mouse can be evaluated by studying Figs. 1 to 8. Fig. 1 (control) is a horizontal section through the right mandibular incisor of a neonatal Webster mouse born of an untreated parent. It is important to note that no cartilage formations are seen in the bony structure that completely surrounds the tooth, although the heavy pink staining in this area indicates that mineralization and calcification are not yet complete. Some of the included cells give the appearance of osteocytes. They are, however, larger than, and do not stain as deeply as osteocytes. The newly formed enamel has apparently been lost through decalcification, indicating a high inorganic content. The cytoplasm of the ameloblasts is homogeneous and contains few vacuoles, and the cell walls are not prominent. They are seen in higher magnification in Fig. 6. The nuclei of these cells stain lightly with hematoxylin, but the nucleoli take a darker stain. Cells of the outer enamel epithelium have formed a stratified layer against the peripheral aspect of the ameloblasts. Tomes's processes can be observed along the inner borders of the ameloblasts. Odontoblasts along the outer pulpal border are well oriented, and some of their processes can be observed as they extend to the dentinoenamel junction. Globular as well as linear calcification is apparent in the dentin layer. Mitotic figures are frequent in the pulpal area.

Fig. 2 is from a section of a lower jaw of a neonatal mouse whose female parent had NaF via drinking water during the last ten days of gestation. Based on a daily average consumption of 3 to 4 c.c. of the 1:50,000 solution, this parent consumed approximately 700 μg of NaF. In comparison with Fig.

Fig. 1.

Fig. 2.

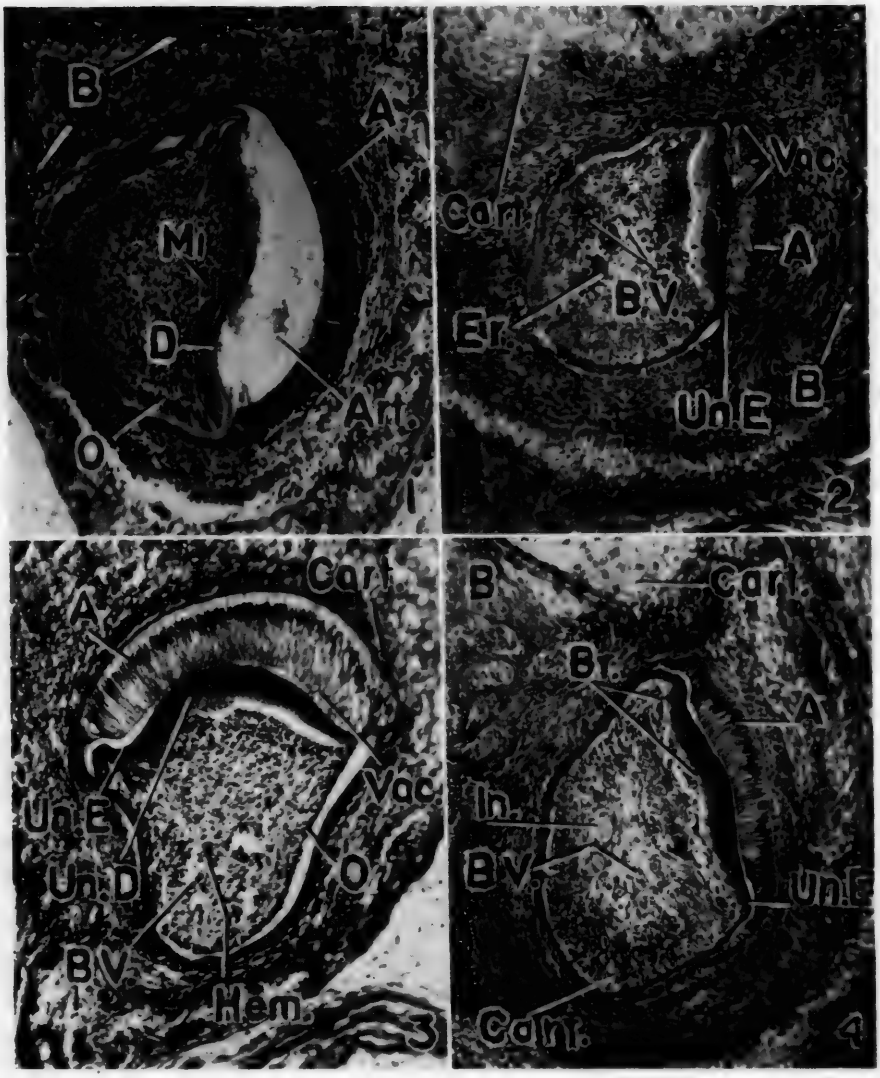

Fig. 3.

Fig. 4.

KEY TO SYMBOLS:

A—Ameloblasts, Art.—Artefact, B—Bone, Br. Bridging of dentin, B. V.—Blood vessel, Cart.—Cartilage, C. W.—Cell wall, D—Dentin, En.—Endothelial cell, Er.—Erythrocyte, Hem.—Hemorrhage, In.—Inclusion, Ly.—Lymphocyte, Mi.—Mitotic figure, N—Nuclei, O—Odontoblast, T. P.—Tomes's process, Un. D.—Uncalcified dentin, Un. E.—Uncalcified enamel, Vac.—Vacuole.

Fig. 1.—Control section, right mandibular incisor of neonatal Webster mouse. (×220; reduced ¼.)

Fig. 2.—Section of right mandibular incisor of a neonatal mouse; female parent received NaF via drinking water for last ten days of gestation. (×220; reduced ¼.)

Fig. 3.—Left mandibular incisor of newborn mouse; female parent received oral CaF_2 during last ten days of gestation. (×220; reduced ¼.)

Fig. 4.—Right mandibular incisor of newborn mouse; female parent received six intraperitoneal injections of NaF during the last week of pregnancy. (×220; reduced ¼.)

Fig. 5.

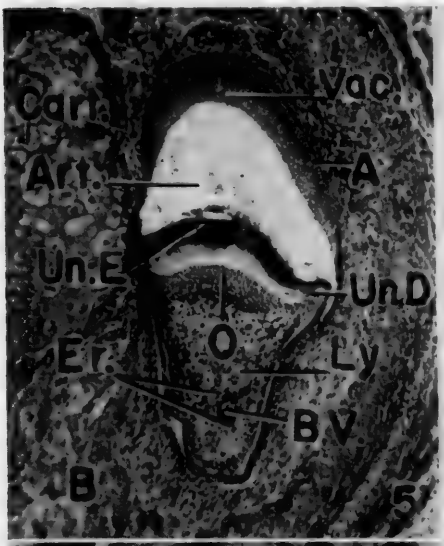

Fig. 6.

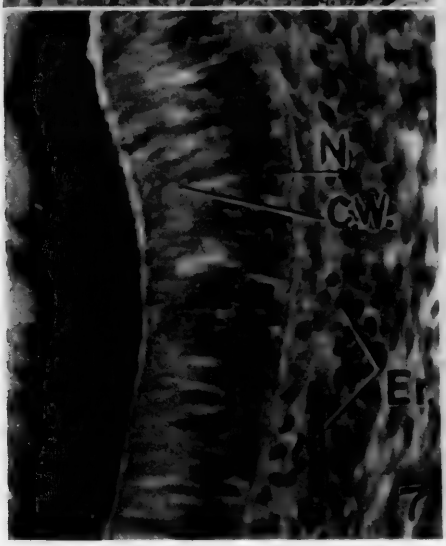

Fig. 7.

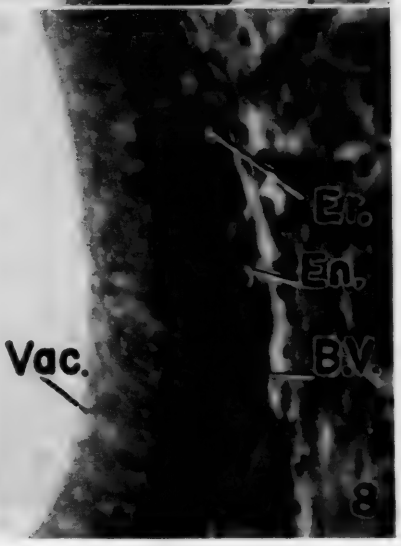

Fig. 8.

(For key to symbols see opposite page.)

Fig. 5.—Right mandibular incisor of newborn mouse; female parent received six daily injections of CaF_2 during last week of gestation. (×220; reduced ¼.)

Fig. 6.—High-power view of Fig. 1. (×650; reduced ¼.)

Fig. 7.—High-power view of Fig. 4. (×650; reduced ¼.)

Fig. 8.—High-power view of Fig. 5. (×650; reduced ¼.)

1, considerable cartilage is evident in the structure surrounding the tooth. Deep staining of the enamel layer and its presence after decalcification indicate a low degree of mineralization. Vacuoles are seen in the cytoplasm of the ameloblasts, whose cell walls appear more definite than those seen in Fig. 1. The entire area appears hyperemic, and numbers of red blood cells in the pulpal area have passed beyond the limits of the vessels in places where the endothelium appears damaged.

Fig. 3 shows a left lower-jaw section of a newborn mouse whose female parent received oral CaF_2 during the last ten days of gestation. About 700 μg of CaF_2 was consumed during this period, which was equivalent to the amount of NaF the female parent received in the previous instance. Large cartilaginous areas are seen about the section of the tooth. Mineralization of enamel and dentin appears similar to that seen in Fig. 2. The pulpal area is hyperemic, and the endothelium of some of these vessels appears disrupted. Here, too, a number of red blood cells lay outside the lumina of vessels. Some ameloblasts have begun to lose their polarity and are not as uniform in cellular structure as those depicted in the control section (Fig. 1). The cell walls of the ameloblasts appear more disorganized in this case than those seen in Fig. 2, but there are not as many vacuoles. Staining of the enamel matrix in this instance was much deeper than in Fig. 2.

Fig. 4 is of a section from the right mandible of a newborn mouse whose female parent received six intraperitoneal injections of NaF during the last week of pregnancy. Amounts given were 0.10 c.c. per injection, making a total of 600 μg of NaF. Again, considerable cartilage is evident in the supporting structure surrounding the tooth. It is also interesting to note that there are a number of cartilage cells along the pulp periphery, with bridging of the predentin matrix with cartilage-like formations. The pulpal blood vessels do not appear to be as damaged as in the previous treated cases, but here too, in places, red blood cells lay beyond the lumina of several of these vessels. In addition, the lumina of several of the main vessels are filled with an eosinophilic-staining homogeneous material or exudate which could indicate a deficient blood supply. Ameloblasts have lost some of their polarity, and their cell walls appear distorted, with many vacuoles appearing in the cytoplasm. They are seen in higher magnification in Fig. 7.

Fig. 5 is from a section through the right mandible of a newborn mouse whose female parent received six daily injections of CaF_2 during the last days of gestation. Here, too, cartilage is evident in the structures surrounding the tooth. Pulpal vessels also appear damaged, which parallels observations in other treated cases. There are great numbers of red blood cells within the lumina of these vessels. Some lymphocytes are also observed in the pulpal area. Ameloblasts have many vacuoles, and their cell walls are disorganized (Fig. 8). Deep staining of the enamel matrix is indicative of its poor mineralization. Globular and linear calcification is apparent in a portion of dentin. The odontoblasts appear well organized.

Administration of CaF_2 and NaF during the whole period of pregnancy seemed to cause resorption of the embryos or fetuses. In these cases, the route of administration was either by injection (0.1 c.c. of 1:1,000 solution daily) or via drinking water (3 to 4 c.c. of 1:50,000 daily). Because of this resorption, administration of NaF and CaF_2 was usually delayed until the last half of the gestation period. It was determined that the female parent could tolerate 1,000 to 1,100 μg of NaF and 600 to 700 μg of CaF_2 during the latter part of gestation without causing death in utero or stillbirths.

DISCUSSION

It has been the objective of this study to report changes in teeeth of newborn Webster miee whose mothers received certain amounts of NaF or CaF_2 during the whole or part of the gestation period.

The report of Knouff, Edwards, Preston, and Kitchen[7] is along the same lines as the present investigation. They administered NaF in different dilutions to three pregnant bitches. In the present study, NaF and CaF_2 were administered via either drinking water or injection to 245 female Webster mice who were at varying stages of pregnancy. Thus, only to a certain extent can comparison be made between their work,[7] where they administered NaF in 25 ppm dilution to one bitch via drinking water, and the present study. They found no marked gross changes in the fetal teeth of the pup, but they did not make histologic sections in this instance. In the present study, where certain groups of mice received either NaF or CaF_2 via drinking water in 20 ppm dilutions, alterations were found in the teeth and jaws of the newborn mouse. This could be determined grossly on cutting, as well as histologically by staining. The difference on gross examination was the ease with which the jaws could be cut, before decalcification, as compared with the controls. Retardation of the calcification of bone and of tooth structures was consistently observed, with the regular appearance of cartilaginous substances in the areas adjacent to the teeth and also within the pulp chambers. In addition, there were structural changes in the ameloblasts and the appearance of cartilage-like cells in the pulp in places where odontoblasts are ordinarily found. In the instance of Knouff, Edwards, Preston, and Kitchen's[7] experiment, where a much larger dose of NaF caused abortion, a marked interference with amelogenesis accompanied by relative increase in dentin formation was observed in the molar teeth. It is difficult to estimate how much fluoride was consumed, for they did not report on the amounts of drinking water consumed daily. In the present study, changes were observed histologically in the structure of the teeth where the female parents had received between 600 and 1,000 μg of fluoride either by intraperitoneal injection or via drinking water. These daily doses were estimated to range between 60 and 100 μg daily. Glock[4] made significant observations on the development of newborn rats whose nursing mothers had NaF added to their diets. She found that for fifteen days after birth there was a continued retardation of development and of bone calcification in these suckling animals. In the present study, where

fluoride were administered during gestation, there was a similar effect on bones as well as on the teeth. Deeper staining of developing bone and teeth in animals from treated parents, as compared with animals from untreated parents, denoted this retardation histologically. Cobb[1] pointed out that the more intense the stain, the more loosely aggregated are the groups in the ground substance, and that faint-staining bone substances indicate a high degree of polymerization. Robinson[13] stated that when cement substance begins to stain metachromatically it has become filled with hydroxy-apatite crystals which make bone hard and give it strength. He further added that processes involved in matrix maturation in normal bone follow one another in rapid succession, but under certain conditions, as in some diseases, various stages may be halted and, thus, steps in these processes may be partly revealed. The presence of cartilage in places where bone should be found, as observed in the present study, appears to indicate that certain steps in the process of bone maturation may have been halted by the presence of the F^- ion available from the NaF or CaF_2 administered to the female parents. Robinson[13] also stated that it is not quite clear whether phosphatase in bone is primarily associated with matrix maturation or crystal formation, or both. Cobb[1] suggested that the enzymes, phosphatase and phosphorylase, exert their chief effect on calcification through the mobilization and orientation of the ground substance of bone. High-energy phosphate is necessary for calcification.[1, 4] The glycolytic cycle which produces these energy-rich phosphate bonds is known to be inhibited or interrupted in at least one place where the F^- ion competes with the Mg^{++} ions as an antimetabolite, namely, in the enolase reaction where fluoride inhibits and magnesium activates. This affords a possible explanation of a mechanism involved in blocking energy requirements for the process of calcification. Thus, energy available allows only for cartilage formation, and not more densely organized substance such as bone or dentin.[2] The presence of cartilage and of cartilage-like cells in pulpal areas where odontoblasts and bone may be found is also indicative of an interference with matrix maturation. In addition, the bridging of newly formed dentin in certain places with cartilaginous formations further supports this concept. Thus, an explanation is offered showing how cartilage and dentin may be intimately associated with each other and are often seen in direct continuity, as was previously observed.[2] These observations on cartilage formation are very similar to those found in rickets, for in such cases cartilage cells are irregular in arrangement, and there is no sharp zone of provisional calcification.[1, 12]

Ingalls,[6] after extensive investigations, observed that at particular times in the development of the embryo or fetus, certain organs take precedence over others in energy requirement. Thus, at a certain time in development, organs such as the teeth require, for future normal development, more energy obtained from metabolic breakdown than other organs at that particular time. Ingalls[6] further noted that metabolic insults, such as anoxia, to pregnant mice resulted in damage to blood vessels in embryos or fetuses. This may have led, in turn,

to energy deprivation in developing organs and ultimately to developmental anomalies. Certain of these findings, particularly those of blood vessel damage, coincide with similar results in this study, for in the present experiment, newborn animals from treated mothers usually had hyperemic blood vessels in the pulpal areas as well as in the areas about the teeth. The senior investigator previously noted increased numbers of red blood cells in the follicles of the incisor teeth of white rats who had been given intraperitoneal injections of CaF_2.[2] Sometimes an eosinophilic-staining exudate filled the pulpal blood vessels in animals from treated mothers, probably denoting an inadequate blood supply. More often these vessels were large or dilated and had prominent endothelial cells. In such cases the endothelium was often disrupted, and red blood cells had passed beyond the lumina, causing small hemorrhages in the pulpal area. Less frequently, this same condition existed in the blood vessels about the tooth, particularly in those vessels adjacent to the outer border of the ameloblasts. Ludford[9] had previously reported that endothelial cells of growing capillaries in tumors seemed particularly sensitive to the toxic action of colchicine and induced extensive hemorrhage. It is interesting to note that both NaF and CaF_2 seemed to exert a similar effect on blood vessels and endothelial cells.

Fluorides, as administered in the present study, seemed capable of passing through the placental membrane and affecting certain structures that were observed in the newborn animal. Six hundred micrograms of CaF_2 and 1,000 μg of NaF presented similar effects but, with increased dosage, toxicity to the neonatal mice seemed greater with CaF_2. In these instances there were more stillbirths and a greater resorption of the embryos or fetuses before birth. This greater toxicity may have been due to the availability of $2F^-$ ions from CaF_2 as compared to 1 from NaF. The female parents also seemed able to tolerate a greater amount of fluorides by daily intraperitoneal injection than by oral administration. This was particularly evident during the months of July and August, when, for some reason, the daily consumption of water was sharply increased even though the animals were housed in a building of constantly controlled temperature and humidity.

SUMMARY

1. Administration of NaF and CaF_2 to the female parents during gestation caused changes in the structure of the jaws and teeth of the neonatal mice. Calcification of bone in the jaws was retarded. There was alteration of the cell structure of the ameloblasts, with retardation of the enamel matrix maturation. Cartilaginous-like formations appeared in the dental pulp. There was a bridging of the dentin matrix with these formations.

2. CaF_2 seemed to be more toxic to the fetuses than NaF, both by injection and by oral administration to the pregnant females.

3. Approximately 600 to 700 μg of CaF_2 and 1,000 to 1,200 μg of NaF were tolerated without causing resorption of fetuses or stillbirths; amounts in excess of this caused these phenomena.

4. In the cases of fluoride-treated animals, the pulpal blood vessels in the teeth of the newborn animals appeared to be hyperemic. There was disruption of the endothelial cells of the vessels, with slight hemorrhaging in the pulpal area as well as in the area immediately surrounding the tooth.

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THE METABOLISM OF FLUORINE IN THE RAT USING F^{18} AS A TRACER

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THE metabolism of minute amounts of fluoride has become a subject of considerable interest in recent years, because of the fluoridation of municipal water supplies as an aid in combating dental caries.[1, 2] At the present time there are few extensive data available on the distribution of small amounts of fluorides in the normal animal body with the exception of that found in the bones, teeth, and excreta.[3-6] One of the major reasons for this lack of data is the difficulty involved in the chemical analysis of fluorine when the amounts present are less than 1 ppm.

Radioactive tracer technics have been used successfully in the study of the metabolism of many elements that are found in the animal body in trace amounts, such as zinc and cobalt.[8, 9] The only useful isotope of fluorine, F^{18}, has a very short half life[10] (112 minutes), which places serious limitations on its effectiveness as a biologic research tool. This isotope emits positrons (0.64 mev.) which result in annihilation radiation (photons).[11] Until recently, investigators who wished to use F^{18} were faced not only with the rapid decay of the isotope, but also with the considerable difficulties encountered in the measurement of the radiations. The measurement either of the positrons, which are absorbed to a large extent in the mass of the samples themselves, or of the annihilation photons, using an ordinary Geiger-Müller counter, is quite inefficient and requires the use of large amounts of radioactive fluorine. To date, there are only four reports of the use of radiofluorine in biologic investigations. Volker, Hodge, Wilson, and Van Voorhis[12] and Volker, Sognnaes, and Bibby[13] determined the uptake of F^{18} in the teeth and bones of the rat thirty minutes after its administration. Wills[14] studied the secretion of F^{18} in the saliva of cats, and Myers, Hamilton, and Becks[15] studied the transfer of F^{18} to the teeth by topical application. With the perfection of the scintillation crystal counter,[16] most of the problems heretofore encountered in the measurement of F^{18} were greatly reduced, the counting efficiency for the photons was substantially increased, and there was thus no longer a need to be concerned with the self-absorption of the positrons in the mass of the samples.

The experiments presented here were designed to shed some light on the following problems: the distribution of F^{18} in the tissues and organs of the normal rat and the possible effects of the route of administration, the presence of moderate (milligram) amounts of stable fluoride, and the age of the animals on this distribution.

From a thesis presented to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bio-Physics.

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EXPERIMENTAL

F^{18} was produced on the sixty-inch cyclotron at the Crocker Laboratory, according to the method described by Myers, Hamilton, and Becks,[15] which was modified to the extent that the salt added was NaCl instead of NaF. Sufficient NaCl was added after the bombardment so that the solution administered to the animals was isotonic. The stock NaCl was assayed for fluorine* and was found to contain an amount of fluoride such that 1.0 ml. of isotonic saline solution prepared from it contained 0.15 μg of stable fluoride. Depending on the volume of the F^{18} solution administered, each animal received stable fluoride in amounts ranging from 0.075 to 0.15 μg. Preparations containing F^{18} and microgram amounts of stable fluoride will hereafter be referred to simply as F^{18}. The technics employed in the measurement of the F^{18} activity of the samples† and in the checking of the radioactive purity of the F^{18} preparations were those described by Myers, Hamilton, and Becks.[15]

(a) *Distribution of Intravenously Administered F^{18} in Young Adult Rats.*—The distribution of F^{18} was studied in young adult female rats of the Long-Evans strain which averaged 185 grams in weight, corresponding to a range in age of 8 to 12 weeks. Subsequently (11 to 17 weeks) rats of this strain undergo notable slowing of growth, and the majority of the epiphyses close.[18] The animals were maintained for at least two weeks prior to use on a pelleted stock diet comparable to "Diet 14" as prepared by the University of California Institute of Experimental Biology.[19] Chemical analysis of the feed showed that it contained 16 ppm of fluoride. On the basis of 15 Gm. of food per rat per day, this would amount to a daily fluoride intake of 1.20 mg. per kilogram of body weight.

F^{18} was administered by injection into the external jugular vein. After injection, the animals were placed in metabolism cages in groups of three to five and were given water‡ but no food.

In order to determine the rate of disappearance of F^{18} from the blood, thirty rats were given F^{18} by vein and were sacrificed one, five, twenty, thirty, forty-five, and sixty minutes after injection. Blood samples were withdrawn by heart puncture, and red blood cells and plasma were separated by centrifugation.

Thirty-seven young adult rats were used in the study of the distribution of F^{18} in normal animals. F^{18} was given intravenously, and the animals were sacrificed according to the following scheme: five rats at fifteen minutes, ten rats each at one and four hours, and twelve rats at nine hours after the injection. At the sacrifice of the animals, blood samples were taken by heart puncture, the animals were weighed, and the pelts were removed. The tissues and organs (as

*The chemical analyses of the stock NaCl and the animal diets for fluorine were made by the firm of Curtis and Tompkins, San Francisco, California.

†All the samples were measured until at least 512 counts had been accumulated. For a total of 512 accumulated counts and a ratio of total counting rate to background counting rate of 1.5 or less, the error introduced because of the statistical nature of particle counting is greater than 10 per cent.[17] When the counting rate of a sample was found to be less than 1.5 times the counter background, the percentage of the administered dose of F^{18} is shown as less than a certain quantity. This quantity is the percentage of the dosage that represents one-half of the counter background.

‡The Berkeley water supply was found to contain no greater than 0.03 ppm of fluorine.[20]

listed in Table I) were dissected out and weighed. The contents were removed from the stomach and intestines, and the empty gastrointestinal segments and their contents were assayed separately. The epiphyses and diaphyses of the leg bones (femur, tibia, and fibula from the right hind leg) were also measured separately. The skinned eviscerated carcass was ground in a meat grinder and assayed in small portions. After assaying, the carcass samples were ashed in a muffle furnace, the soft tissue ash was removed with water, and the ash weights of the skeletons were determined. The small tissues and those for which it was anticipated that the F^{18} content would be low were pooled to increase the weighing and counting accuracy. These were pancreas, brain, spleen, parotid glands, lacrimal glands, cervical lymph nodes, adrenals, and cartilage (from the xiphoid process).

TABLE I. THE DISTRIBUTION OF F^{18} IN YOUNG ADULT RATS FIFTEEN MINUTES, ONE, FOUR, AND NINE HOURS AFTER INTRAVENOUS ADMINISTRATION; VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT

	¼ HOUR		1 HOUR		4 HOURS		9 HOURS	
	% PER ORGAN	% PER GRAM	% PER ORGAN	% PER GRAM	% PER ORGAN	% PER GRAM	% PER ORGAN	% PER GRAM
Molars	—	—	0.44	3.04	0.41	2.75	0.30	2.50
Incisors	—	—	1.02	3.14	1.13	3.98	1.33	3.61
Skeleton	32.9*	—	55.9	—	64.0	—	56.0	—
(Mandibles)†	—	—	2.56	5.08	2.97	5.96	2.39	4.61
(Leg bones)†	2.80	1.76	5.05	3.97	5.87	4.33	4.94	3.31
Muscle	19.4	0.19	6.55	0.08	1.06	0.01	0.55	0.006
Skin	11.2	0.29	2.00	0.07	0.25	0.008	0.17	0.005
Blood	8.05	0.50	0.99	0.08	0.12	0.008	0.06	0.005
Cartilage	—	0.31	—	0.37	—	0.16	—	0.26
Spleen	0.50	0.52	0.06	0.06	0.009	0.009	0.003	0.003
Lung	1.13	0.58	0.19	0.11	0.10	0.04	0.06	0.03
Liver	4.85	0.49	0.49	0.06	0.28	0.03	0.04	0.005
Kidney	1.46	0.84	0.27	0.18	0.04	0.03	0.03	0.02
Stomach	0.27	0.32	0.05	0.07	0.009	0.009	0.004	0.003
Stomach contents	0.06	—	0.08	—	0.006	—	0.001	—
Small intestine	1.11	0.28	0.24	0.07	0.03	0.009	0.02	0.004
Small intestine contents	1.73	—	1.26	—	0.14	—	0.03	—
Large intestine	0.41	0.35	0.06	0.07	0.03	0.04	0.01	0.006
Large intestine contents	0.20	—	0.09	—	0.84	—	0.48	—
Cecum and contents	0.42	—	0.32	—	1.27	—	0.30	—
Salivary gland	—	1.23	—	0.08	—	0.01	—	0.005
Lacrimal gland	—	0.37	—	0.04	—	0.01	—	0.004
Lymph node	—	0.64	—	0.01	—	0.008	—	0.004
Brain	—	—	0.02	0.02	0.01	0.009	0.003	0.004
Pancreas	—	0.41	—	0.04	—	0.009	—	0.002
Thyroid	0.001	—	0.002	—	0.001	—	0.004	—
Adrenal	0.04	—	0.004	—	0.001	—	0.003	—
Balance	6.09	—	11.6	—	7.20	—	7.54	—
Urine	9.75	—	17.2	—	22.6	—	31.4	—
Feces	0.04	—	0.98	—	0.46	—	1.83	—

*Includes teeth.
†Included in skeleton.

(b) *The Distribution of Orally Administered F^{18} in Young Adult Rats.*— In order to ascertain whether or not the distribution of F^{18} in normal animals might be affected by the route of administration, tracer studies were also undertaken in which F^{18} was given orally. Fifteen young adult rats that had been

fasted overnight were given F^{18} by stomach tube and were sacrificed in groups of five at intervals of one, four, and nine hours after administration. The tissues and organs assayed were the same as in Part a except that the teeth and mandibles were not taken.

(c) *The Effect of Milligram Amounts of Stable Fluoride on the Distribution of F^{18} in Young Adult Rats.*—Each of eight young rats was given F^{18} and 10 mg. per kilogram of fluoride ion as isotonic NaF intravenously. Five of the animals were sacrificed fifteen minutes after the injection, and the other three were sacrificed nine hours after the injection. The tissues and organs taken were the same as in Part b.

(d) *Studies on the Distribution of Intravenously Administered F^{18} in Mature Rats.*—The animals in this group averaged 250 grams in weight and were 25 weeks old when used. Prior to use they were maintained for four and one-half months on a semisynthetic diet comparable in composition to the stock diet mentioned in Part a. Chemical analysis showed that the semisynthetic diet contained 8 ppm of fluoride, corresponding to a daily fluoride intake of 0.5 mg. per kilogram of body weight. In all, thirteen mature animals were used. Groups of four animals were sacrificed at intervals of one and four hours after intravenous injection of F^{18}, and a group of five animals was sacrificed nine hours after the injection. The tissues and organs studied were as shown in Table II.

(e) *Radioautography With F^{18}.*—When it was found from the tracer studies previously described that the F^{18} concentration in the epiphyses of the leg bones was greater than that in the diaphyses, it was considered advisable to prepare radioautographs of the distal ends of the femurs in order to determine the initial site of deposition of F^{18} in bone. The distal end of the femur was chosen because the epiphysis does not close until the animals are approximately 1,000 days old.[18] Because of the short half life, it was necessary that fresh material be used, and the technics employed, relatively crude. The femurs were removed from five young adult rats and five mature rats one hour after the intravenous injection of F^{18}. The fresh bones were cut lengthwise with a jeweler's saw and were placed cut side down on a piece of Eastman-Kodak no-screen x-ray film. In order to prevent the bones from sticking to the film and to avoid chemical fogging of the film, a thin sheet of mica (2 mg. per square centimeter) was placed between the bone and the film. The bones and film were placed in a light-tight box lined with sponge rubber, and were weighted down to maintain good contact during exposure.

The exposure varied from five to ten minutes depending on the amount of F^{18} injected. The bones were then fixed in absolute alcohol for forty-eight hours and were stained with silver nitrate according to the method described by McClung.[21]

The amounts of fluoride present in the diets of both the young adult and mature rats were considered far below the level of toxicity. Rats have been maintained on these diets in this laboratory for long periods of time without any evidence of retardation of skeletal growth or any discernible dental effects.

TABLE II. THE DISTRIBUTION OF F^{18} IN NORMAL MATURE RATS ONE, FOUR, AND NINE HOURS AFTER INTRAVENOUS ADMINISTRATION; VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT

	1 HOUR		4 HOURS		9 HOURS	
	% PER ORGAN	% PER GRAM	% PER ORGAN	% PER GRAM	% PER ORGAN	% PER GRAM
Molars	0.26	1.68	0.26	1.64	0.18	1.33
Incisors	1.18	2.72	1.40	3.12	1.38	3.01
Skeleton	47.2	—	52.8	—	48.5	—
(Mandibles)*	2.00	2.94	2.04	3.14	2.10	3.22
(Leg bones)*	3.78	2.32	4.34	2.74	3.97	2.46
Muscle	6.81	0.06	0.88	0.008	0.47	0.004
Skin	3.58	0.08	0.62	0.01	0.57	0.01
Blood	1.41	0.08	0.15	0.008	0.08	0.004
Cartilage	—	0.20	—	0.12	—	0.17
Liver	0.48	0.06	0.06	0.008	0.04	0.005
Kidney	0.26	0.15	0.04	0.02	0.02	0.01
Stomach	0.08	0.06	0.02	0.01	0.009	0.008
Stomach contents	0.12	—	0.01	—	0.003	—
Small intestine	0.17	0.06	0.03	0.008	0.02	0.004
Small intestine contents	3.26	—	0.22	—	0.04	—
Large intestine	0.06	0.04	0.02	0.01	0.02	0.01
Large intestine contents	0.19	—	0.23	—	1.65	—
Cecum and contents	0.42	—	0.94	—	0.95	—
Salivary gland	—	0.08	—	0.009	—	0.003
Lacrimal gland	—	0.07	—	0.01	—	0.003
Lymph node	—	0.06	—	0.009	—	0.005
Thyroid	0.001	—	0.001	—	0.001	—
Adrenal	0.004	—	0.001	—	0.001	—
Balance	5.04	—	13.5	—	10.6	—
Urine	29.1	—	28.5	—	36.0	—
Feces	0.08	—	0.26	—	0.93	—

*Included in skeleton.

At no time did the amount of F^{18} administered to any animal exceed 4.0 μc per gram of body weight. In view of the short half life of this isotope and the short-term nature of the experiments, damage to the tissues of the animals and the consequent impairment of their functions due to internal irradiation were considered unimportant.

The total amounts of F^{18} present in blood and muscle were calculated using the figures obtained for the per cent of F^{18} per milliliter of whole blood and per cent per gram of muscle, and assuming that these two tissues corresponded to 7 per cent and 45 per cent of the total body weight, respectively.[22] The F^{18} content of the edentulous skeleton was estimated from the per cent of F^{18} per gram of fresh bone obtained from the leg bone samples, and the calculated skeletal weight, which was based on the ash weight of the skeleton. The ash weights of the leg bones of ten young adults and thirteen mature rats were determined as follows: 36.6 ± 0.7 per cent ash content of fresh (not defatted) young adult bone and 34.2 ± 0.5 per cent ash content of fresh mature rat bone.

The per cent of the administered F^{18} in the muscle, bone, blood, and cartilage samples was added to the per cent of dose obtained for the skinned eviscerated carcass. From this "total carcass" were subtracted the values calculated for total blood, muscle, and skeleton. The remainder, which varied considerably (2.8 to 13.5 per cent of the administered dose), has been desig-

nated "balance." This consisted of tissues such as connective tissue, cartilage, glandular tissue, lymphoid tissue, fat, nervous tissue, and blood vessels. The size and variability of the balance are probably due, in part, to the use of an average figure for the ash content of bone in computing the skeletal wet weights. A certain amount of error is also introduced in assuming that the leg bones represent an accurate index of the ability of the skeleton, as a whole, to accumulate F^{18}.

The results of the tracer studies described here were corrected for deviations of recovery of the injected F^{18} from 100 per cent. The actual experimental recoveries for individual animals varied from 84.5 per cent to 117 per cent of the administered dose because of pooling of excretions and loss of material in the meat grinder which was used to prepare the carcasses. The average recoveries for each experimental group of three to five rats did not vary quite as much and were generally between 85 per cent and 95 per cent of the administered dose.

RESULTS AND DISCUSSION

It is apparent from Fig. 1 that F^{18} administered intravenously in the form of a salt, NaF, disappears quite rapidly from the blood. The nature of the curve shown suggests that it may be a composite of several individual curves and possibly the result of such processes as the equilibration of the F^{18} in the extracellular and intracellular fluids, skeletal deposition, and urinary excretion. From Fig. 1 and Table I it can be seen that an equilibrium was rapidly established between the F^{18} in the blood and in most of the tissues. The time necessary to attain this equilibrium appears to depend upon the blood supply of the individual tissues and organs. The F^{18} concentrations expressed in per cent of administered dose per gram of wet tissue of the relatively vascular tissues, such as liver, spleen, and small intestine, were very nearly the same as the F^{18} level in the blood fifteen minutes after the injection. The F^{18} concentrations in skin and muscle, tissues that normally have a relatively slower circulation, were considerably below the blood level at this time, although equilibrium was reached at the end of one hour. The F^{18} concentrations of the soft tissues, with the exception of kidney and salivary glands, very nearly paralleled the blood level at all the time intervals investigated.

The plasma was found to contain nearly twice as much F^{18} as the red blood cells, giving a ratio, RBC: plasma, 0.54 ± 0.02, a value similar to those obtained for the other halogens. For chloride and bromide ions, the ratio in human beings is 0.51,[23] and for iodide ion it is 0.67.[24] These findings and the paralleling of the F^{18} concentrations in the soft tissues with the blood level indicate that cells are as freely permeable to fluoride ion as they are to the ions of the other halogens.

It will be noted that fifteen minutes after the injection, while the blood level of F^{18} is high, the F^{18} concentration in the parotid glands is a little more than twice that of the blood. This observation is in accord with the findings of Wills[14] and Volker, Sognnaes, and Bibby[13] on the presence of F^{18} in the saliva of rats and cats a short time after intraperitoneal injection. However, since the

salivary glands of all the animals sacrificed at this time were pooled, this value represents only a single determination and cannot be considered quantitative.

It is worthy of note that the thyroid did not accumulate F^{18} to any significant extent. This is interesting in view of the work of other investigators on the thyroidal uptake of other members of the halogen group.[25, 26] The F^{18} concentration in the thyroid (calculated on the basis of a normal rat thyroid weight of 20 mg.) did not exceed the blood level at any time during these experiments.

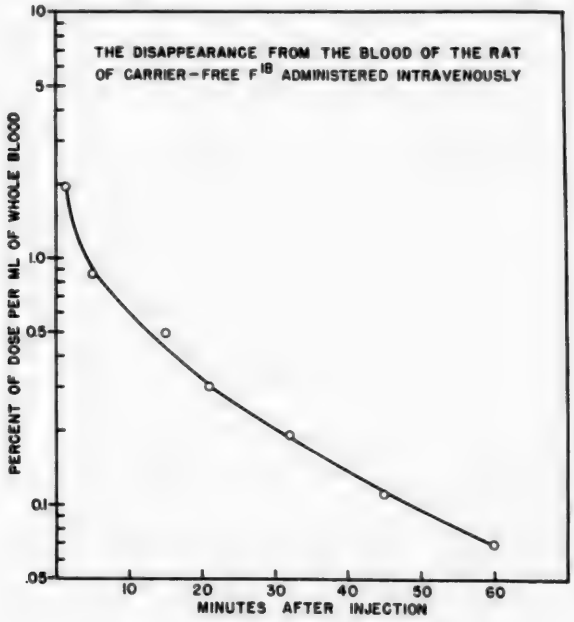

Fig. 1.

The kidney was the only soft tissue that consistently showed a higher F^{18} concentration than the blood. This observation is reasonable since the excretion of fluorine is principally by way of this organ. The excretion of F^{18} in the feces to a small extent seems fairly well established by these experiments. Although most of the F^{18} that made its way into the contents of the gastrointestinal tract following intravenous injection was apparently reabsorbed, some was also found in the feces. The possibility that the F^{18} in the feces was due to urinary contamination seems disproved by the fact that F^{18} was found in the formed feces in the large bowel.

The major site of F^{18} deposition was in the teeth and bones and to a lesser extent in the cartilage. (As was the case with the salivary glands, the cartilage samples were pooled, and therefore the figures shown for the F^{18} concentration in cartilage should be regarded with caution.) F^{18} was rapidly accumulated by the bone and reached a peak value in the leg bones and mandibles about four

hours after the injection. The F^{18} content of the diaphyses of the leg bones was such that the ratio of the F^{18} content, diaphyses: epiphyses, was 0.45 at fifteen minutes after the injection, decreasing to 0.35 for the rest of the time intervals. On the basis of decreasing F^{18} concentration, the bony tissues sampled can be arranged in the following order for all of the time intervals studied: mandible, leg bone epiphyses, leg bone diaphyses. This indicates that a short time after its administration, nine hours or less, the amount of F^{18} taken up by a bone depends in large measure on its vascularity, substantiating an observation by Volker, Sognnaes, and Bibby.[13]

In general, the concentrations of F^{18} in the teeth were lower than in the leg bones at the corresponding time intervals. The incisors followed the same general pattern as the other bony tissues, showing a rapid initial F^{18} uptake, and a peak concentration four hours after the injection, which gradually decreased during the ensuing five hours. On the other hand, the molars showed a peak F^{18} concentration at one hour and a gradual decline thereafter.

The absorption of F^{18} from the gastrointestinal tract after oral administration was quite rapid. Seventy-five per cent of the administered dose was absorbed in the first hour, and 90 per cent by the end of nine hours. The data on the oral administration of F^{18} were computed on the basis of per cent of dose absorbed, and the values thus obtained for the distribution of F^{18} in the blood and soft tissues were very nearly the same as in the intravenous studies. The skeletal uptake of orally administered F^{18} as a soluble salt was 60.9 per cent at one hour and 67 per cent at four hours, slightly higher than when F^{18} was given by vein. During this time, the urinary excretion of F^{18} was somewhat reduced, 18.2 per cent at four hours. However, after nine hours, the skeletal deposition, 56.5 per cent, and urinary excretion, 28.7 per cent, were very nearly the same as the values obtained in the intravenous experiments. There was more F^{18} present in the feces of the animals which received F^{18} by stomach tube, 4.14 per cent of the dose at nine hours, indicating that absorption of microgram amounts of fluoride ion may not be complete.

When 10 mg. per kilogram of fluoride as isotonic NaF was given simultaneously with the essentially carrier-free preparation of F^{18}, the animals received nearly 20,000 times more fluoride than did the animals which received only the standard F^{18} preparation. Several notable differences were observed between the two groups, at the fifteen-minute interval. The major differences found at this time between the animals that received F^{18} alone and those receiving added stable fluoride can be summarized as follows: the animals receiving added carrier showed a considerable higher F^{18} content in the kidneys (3.34 per cent), in the thyroid (0.006 per cent), and slightly higher F^{18} concentrations in the blood and soft tissues (0.28 per cent per gram of muscle and 0.65 per cent per milliliter of blood). The F^{18} content of the leg bones was higher, 4.22 per cent, and the urinary excretion was lower, 1.14 per cent. Nine hours after the injection, the distribution and excretion patterns of the F^{18} and F^{18}+ stable fluoride groups were completely comparable, indicating that (within certain

limits*) fluorides are eventually metabolized in the same fashion regardless of the size of the dose, but with a little more difficulty when larger doses are given.

In the tracer studies in which F^{18} was given in the essentially carrier-free state, there was actually sufficient stable fluoride present so that a 200-gram rat received 0.5 μg of fluoride per kilogram of body weight. The longest time interval investigated in these experiments was nine hours after injection. However, since by this time F^{18} was being eliminated from the skeleton only gradually, the majority of the F^{18} found in the skeleton at this time can be considered stored. In McClure's[27, 28] balance studies on young adult men, he claimed that there was no storage of fluoride when the daily intake was of the order of a few milligrams. The daily intake in his studies can be calculated as approximately 53 μg per kilogram, an amount nearly 100 times greater than was used in the tracer experiments described here. The results of these studies, together with those of Hodge,[29] Savchuck and Armstrong,[30] and Glock, Lowater, and Murray,[31] who found that the skeletal fluoride content increased slowly with age on diets low in fluoride, indicate that apparently there is no level of fluoride intake below which storage ceases and excretion is complete, as was stated by McClure.

The distribution of F^{18} in the mature rat is shown in Table II. By and large there were few significant differences between the distributions of F^{18} in young adult and mature rats. In the mature rats, the urinary excretion was slightly greater, as was also the F^{18} found in the contents of the gastrointestinal tract.

The values obtained for the F^{18} content and F^{18} concentration of the molars of the mature rats were considerably lower than the corresponding values found for the young adults. This difference between the molars of young and mature animals in their ability to accumulate F^{18} seems to be a function of the decrease in pulpal activity and calcification of the molars with increased age.[32]

Since the rat incisor continues to form and erupt throughout life, it was not surprising that the incisors of the mature animals, although heavier than those of the young adults, accumulated F^{18} to the same extent as did the young animals.

It was found that the skeletons of the mature rats accumulated less F^{18} than did the skeletons of the young adults. The pattern of uptake, at first quite rapid to a peak value at about four hours followed by a gradual decline, was the same regardless of age or the amount of fluoride administered. However, in the mature rats the peak was less sharply defined, the rise was more gradual, and the ensuing loss appeared slightly smaller. It was found that after nine hours the skeletons of the young rats retained 56 per cent of the administered F^{18} while the mature skeletons retained only 48 per cent. These findings are in accord with the work of Copp, Hamilton, Jones, Thomson, and Cramer,[33] who showed that the turnover of the mineral components of bone was much more rapid in growing animals, and of Savchuck and Armstrong,[30] who found that the retention of fluoride in the skeleton was greater in young rats (53 per cent as opposed to only 36 per cent for mature rats).

*Animals that were given 14 to 17 mg. of fluoride per kilogram intravenously in pilot experiments died within one hour after the injection.

Figs. 2 and 3 show the distal end of the right femur and the corresponding F^{18} radioautograph that was prepared from one of the young rats. Since radioautographs of bones of mature rats showed the same general distribution of F^{18} as seen in the young adults, they are not illustrated.

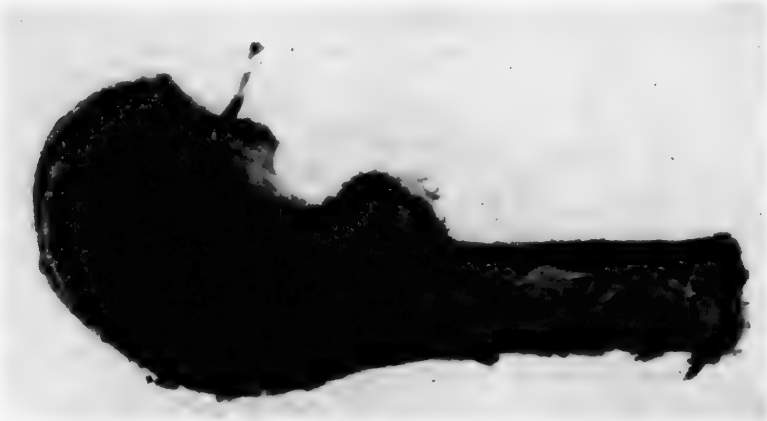

Fig. 2.—The silvered surface of the distal end of the right femur of a young adult rat 75 days old.

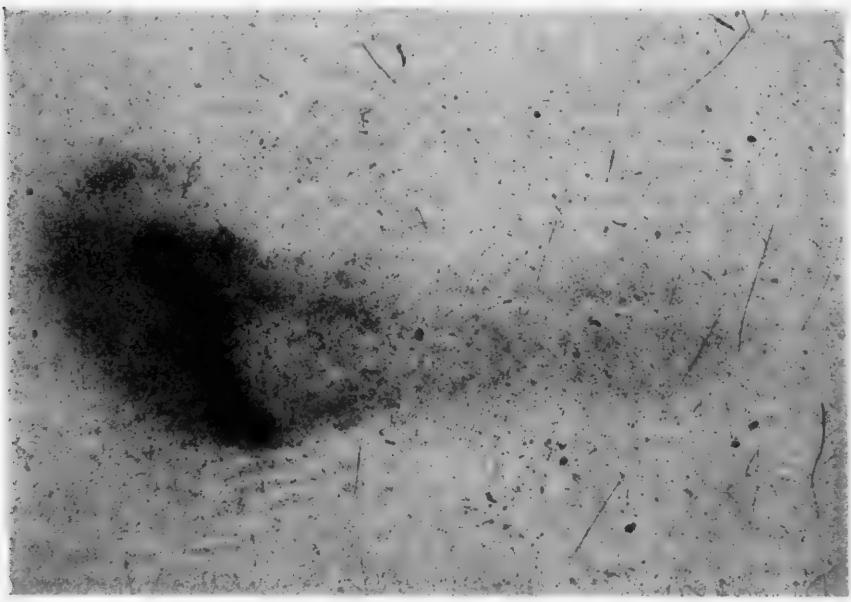

Fig. 3.—The F^{18} contact radioautograph of the bone surface shown in Fig. 2.

Despite the poor resolution obtainable from such relatively crude methods as were used in the preparation of these radioautographs, several generalizations can be made about the initial deposition of F^{18} in the bones of the rat. The area of greatest F^{18} concentration one hour after administration was in the

region of the epiphyseal cartilage, probably in the most recently calcified bone just below the epiphyseal plate. The spongy bone of the epiphysis and the upper end of the diaphysis, especially on the front side, were the next most heavily blackened areas. This was not quite as apparent in the radioautographs made from the young bones as it was in the mature bones. There appears to be little if any F^{18} in the marrow cavity.

SUMMARY

Radioactive tracer and radioautographic technics using NaF tagged with F^{18} were employed to study the metabolism of soluble fluoride ion in rats. Young adult females and mature females were used for these studies. F^{18} was administered orally and by intravenous injection in an essentially carrier-free state and with added fluoride carrier. Radioautographs were prepared from the femurs of both young and mature rats. The results permit the following conclusions:

1. Cell membranes appeared to be freely permeable to F^{18} when it was administered as NaF.

2. There did not seem to be any significant deposition of F^{18} in any of the soft tissues of the rat, with the possible exception of the kidney, when it was given with stable fluoride in amounts ranging from 0.5 μg to 10 mg. per kilogram of body weight.

3. Urinary excretion and skeletal deposition of F^{18} were similar regardless of whether the stable fluoride dose was 0.5 μg or 10 mg. per kilogram, indicating that there was no level of fluoride intake below which all ingested fluoride would be excreted.

4. There appeared to be genuine fecal excretion of F^{18}, although the amount excreted by this route was quite small.

5. Absorption of orally administered F^{18} was not complete nine hours after its administration. At this time there was about 10 per cent of the administered dose in the cecum, large intestine contents, and feces.

6. The distribution of F^{18} in the rat was essentially the same whether it was given orally or by vein.

7. The distribution of F^{18} in the soft tissues of the rat was not significantly affected by the age of the animals. In contrast, the F^{18} uptake of the teeth and bones was definitely lower in mature rats than in young adults.

8. The extent to which a bone will accumulate F^{18} seemed to depend upon the vascularity of the bone and on its growth activity.

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EFFECT OF CHEWING ON THE PHOSPHORUS EXCHANGE IN TEETH

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THIS study attempts to determine whether any changes occur in the phosphorus turnover in teeth owing to the degree of functional stress that they are exposed to during mastication. It is principally and in vivo study although certain controls were carried out after extraction of teeth in order to check on background influences in the mouth.

Following the original observation by Chiewitz and Hevesy,[1] that phosphorus ions in the maturing and mature tooth are being continually replaced, further studies attempted to define the nature and rate of this turnover. The experiments by Hevesy, Holst, and Krogh[2] disclosed the distribution of the active phosphorus turnover in the different component tissues of the teeth, and Volker and Sognnaes[3, 4] and Pedersen and Schmidt-Nielson[5] indicated the pathways of access of the labeled phosphorus to the dental tissues.

The turnover rate in powdered dentin and enamel, both fresh and ashed, was described by Neuman and Mulryan[6] and by Underwood and Hodge[7]; various exchange patterns were found to exist, and differences were observed in the phosphorus uptake between young and old,[8-10] growing and nongrowing,[2] and more- and less-mineralized tissues,[10] and among the various components constituting the tooth.[2]

Factors such as age influence or exact localization of the P^{32} deposition were not explored in the present project. The observations were limited to any differences occurring between the more- and the less-functioning half dentitions in the same animal. In order to obtain a sizable contrast in the degree of function of the teeth to be measured, we selected various methods of removing functional stress from one half dentition in rhesus monkeys and burdening the task of mastication entirely or as far as feasible to the other half.

Measurement of Phosphorus Turnover in Vivo.—In most of the work with radioactive indicators, the tissues under investigation are removed from the organism. Such procedure is largely necessary to calculate a ratio of weight to radioactivity, in order to obtain absolute values. If relative values, however, are the aim of the investigation, as in a left and right comparison under different physiologic conditions or in a comparison under physiologic and pathologic circumstances within the same organism, then the radiation emitted by an active isotope may be measured topically at points accessible to measuring instruments. The enamel is more ideally suited to these direct measurements than most other tissues in the body. If a pure beta emitter is used, counts by contact with the occlusal surface can be obtained with a small end-window tube instrument.

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Topical measurement of in vivo concentration of radioactivity[11-13] has been applied to the thyroid, the breast, and the eye, although because of their penetrating power, mostly gamma ray-emitting isotopes are being used for all but the most superficial layers of the body. In contrast, in the case of the enamel, the selection of a pure beta emitter is a distinct advantage, as only the rays emitted by the superficial layers will reach the counter because of the low penetration of the radiation. Such a study is further facilitated by the fact that localization and sufficient shielding of the background can be achieved readily with pliable lead foil.

For our measurements on the enamel surfaces we used a Geiger-Müller tube of pencil shape,* small enough to be introduced into the mouth of a rhesus monkey. The very thin end window of the tube corresponds in size to the occlusal surface of a single molar.

After adequate shielding, the Geiger tube window was covered by a thin membrane of cellophane sheeting 7/10,000 of an inch thick to avoid contamination of the instrument. This cellophane was changed with every measurement. In order to avoid interference with saliva and oral debris, the teeth were scaled, polished with pumice, flushed with water, and dried with cotton before measurements were made. However, the activity of saliva in thin layers was found to be negligible compared to the enamel activity. The cellophane-covered instrument was then brought into direct contact with the occlusal surface of the tooth.

The method allows one to follow the trend and rate of phosphorus exchange in the enamel from hour to hour, from day to day, and over a period of weeks without necessitating extractions, and it permits the observation of any variations in the turnover pattern under varying physiologic and pathologic conditions. An animal may be observed repeatedly under similar experimental circumstances.

Preliminary counts of the contralateral molars were obtained on untreated monkeys, and no significant difference was found to exist in the phosphorus uptake in the left and right quadrants. Comparing upper and lower corresponding teeth, slightly lower counts were found fairly consistently on the lower molars as compared with the upper molars in the same animal.

The estimations on the experimental animals were carried out on molars and premolars. The findings were in a similar direction on the examined teeth, and only the observations on the first lower molars will be described in detail. In all experiments radiophosphorus was injected intraperitoneally, in order to make use of the primary phosphorus metabolism in the liver.

Unilateral Dental Disuse Induced by Nerve Resection.—In a rhesus monkey, weighing 6 kilograms, ½ mc. of P^{32} was injected intraperitoneally two years after this animal had a unilateral intracranial section of the third division of the fifth cranial nerve. Observation of chewing motions and of the dentition indicated that chewing had been confined to one side from the time of the nerve resection.

*222 tube, Anton Lab. Mfg. Co.

Counts were obtained from the occlusal surface of the molar teeth at one-half hour intervals for the first six hours, and weekly measurements were made subsequently (Fig. 1).

The counts in the nerve-resected animal obtained from the corresponding first lower molars were unequal. The differences were statistically significant and were consistently found after repeated administration of P^{32}.

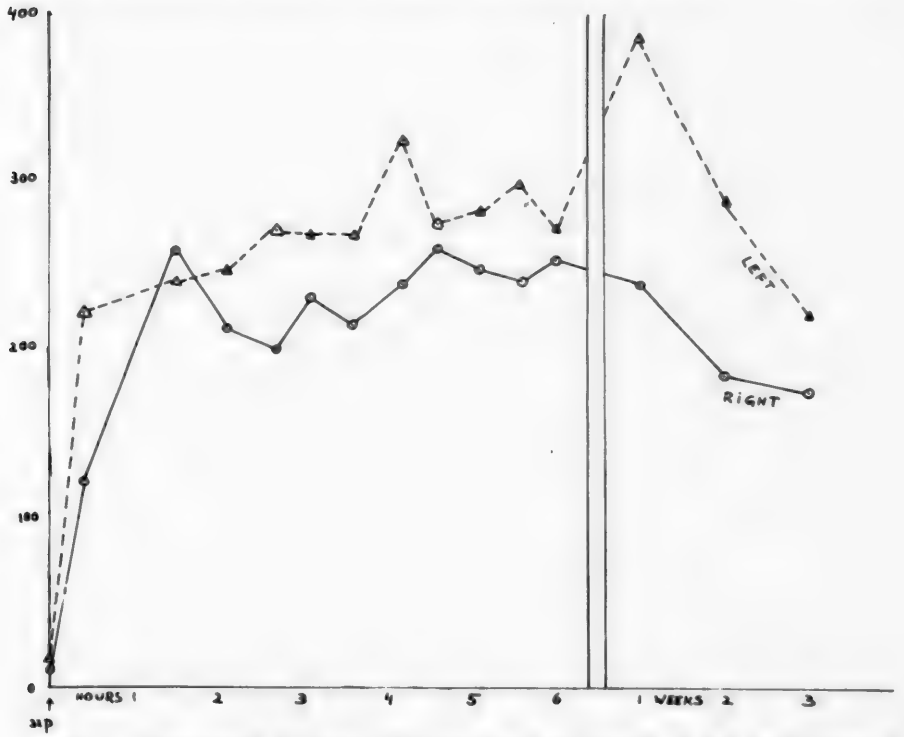

Fig. 1.—Radioactivity in counts per minute from the surface of first lower molars in situ, after left fifth cranial nerve resection. Values for one, two, and three weeks following injection of P^{32} are corrected for decay of radioactivity.

This experiment was designed to compare a functioning with a nonfunctioning half dentition. It is difficult to determine, though, how much of the apparent differences can be attributed to a variation in the function of the two jaw halves alone, as sensory nerve fibers were unavoidably cut with the motor fibers. Furthermore, interference with sympathetic fibers may have influenced the salivary glands as well as regional circulation, all factors with a potential bearing on the phosphorus uptake. Although the findings were consistent and significant, it was deemed advisable to vary the experimental conditions in assessing the results, by dissecting and removing the three major masticatory muscles on one side on another monkey.

Unilateral Dental Disuse Induced by Muscle Resection.—Under general anesthesia the masseter, temporalis, and internal pterygoid muscles of a 4-year-

old rhesus monkey were dissected and removed. After a period of three weeks and for six months following, measurements were taken under the same experimental conditions as on the nerve-resected monkey; a dose of 500 μc of radioactive phosphorus for a monkey weighing 6 kilograms was injected intraperitoneally. This dose was repeated in intervals of forty-five days.

Observations within the first three months following the operation indicated a similar trend of an increased P^{32} uptake on the operated side. However, in subsequent measurements the findings were not consistent and not considered to be statistically significant.

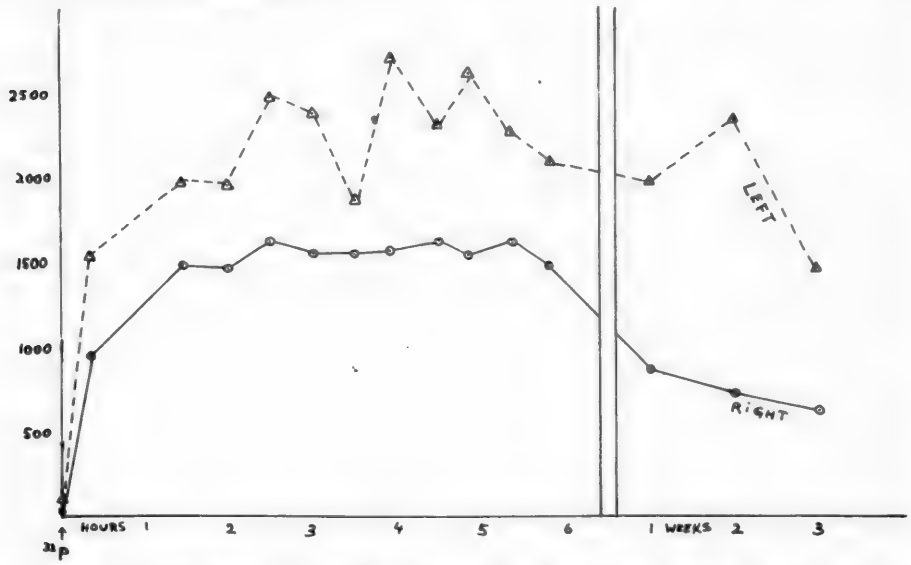

Fig. 2.—Radioactivity in counts per minute from the surface of first lower molars in situ, after removal of upper left quadrant of teeth. Values for one, two, and three weeks following injection of P^{32} are corrected for decay of radioactivity.

The degree of nonfunction achieved by the method of muscle resection appeared to be only moderate. Ability to chew on the operated side returned, probably because of hypertrophy of nonresected strands and muscles. Regeneration seemed to set in at once, and it was again held advisable, in appraising the results, to alter experimental conditions, this time by removing unilaterally the upper teeth in rhesus monkeys, and to compare the phosphorus retention in the functioning and nonfunctioning lower molars.

Unilateral Disuse Induced by Quadrant Resection.—In a further attempt to narrow down the variables to the factor of function, the upper left quadrant of teeth was removed from each of two rhesus monkeys under general anesthesia. Before the surgical intervention, labeled phosphorus was injected intraperitoneally, and the number of disintegrations measurable topically on the occlusal surface of the molars was compared for both jaw halves. On repeated examinations and measurements, no significant differences were found for the P^{32} absorption in contralateral teeth.

Four weeks after the quadrant resection, topical counts were again taken at frequent intervals, first half-hourly after P^{32} injection, then daily and weekly. Radiophosphorus was reinjected after eight and twelve weeks, and the measurements were repeated (Fig. 2).

The findings after quadrant resection were similar to those produced by nerve resection. The degree of the differences appeared to exceed the variations found by the previous methods.

P^{32} Variance in Extracted Teeth After Unilateral Disuse.—In animals with unilaterally functioning teeth, inflammatory changes on the gingivae opposing the edentulous quadrant were observed frequently. The phosphorus uptake and retention in these mucosal tissues were often higher than on the normally functioning side. Increased P^{32} uptake in tissue with inflammatory changes has been described,[12] and the possibility of background influence from the mucosa on topical measurements, despite shielding, had to be considered. Therefore, in the two monkeys in which the upper left quadrant of teeth had been extracted three months previously, both lower first molars were removed one week following the intraperitoneal injection of 500 μc of P^{32}.

Subsequently each tooth was weighed and digested in concentrated hydrochloric acid. The resulting solution was diluted to 25 c.c., and aliquots were taken for measurement of radioactivity using the M-6 counter tube described by Veall.[14] Activity was determined in counts per minute per gram of tooth.

TABLE I

RELATIVE RADIOACTIVITY OF FUNCTIONING AND NONFUNCTIONING MOLARS DETERMINED BY ACID DISSOLUTION OF WHOLE TOOTH

MONKEY	TEETH	COUNTS PER MIN. PER GRAM OF TOOTH		PER CENT DIFFERENCE
		LEFT (NON-FUNCTIONING)	RIGHT (FUNCTIONING)	
Q1	First permanent molars	9,890	8,880	10.2
Q1	First and second deciduous molars, pooled	5,490	4,850	11.7
Q2	First permanent molars	418	236	43.5

These figures represent the activity in the entire tooth, while the topical measurements from the occlusal surface indicated the enamel activity only.

The fact that penetration of beta radiation from the dentin was negligible was established by gradually grinding down a molar from the root to the occlusal surface. Values in topical measurement from the surface remained uninfluenced by the bulk of the tooth or by its gradual disappearance, until a thickness of less than 1 mm. was reached.

DISCUSSION AND CONCLUSIONS

Comparing the results obtained by different methods, it appears that there exist differences of P^{32} uptake and retention in the molars, depending on function and nonfunction. The process involved cannot readily be explained because of the apparent incongruity of an increased P^{32} uptake and retention in the nonfunctioning molars. In sifting the findings on other tissues, we meet a

similar phenomenon in the muscle. Artom[15] found that denervation was followed by a pronounced increase in the capacity of the muscle to deposit labeled phosphorus. These observations were confirmed by Friedlander, Perlman, and Chaikoff,[16] who also noted an increase in the uptake of P^{32} in muscles after nerve resection.

Parallel to the observations on the teeth in relation to disuse, we immobilized one limb of a rhesus monkey by applying a plaster cast for a period of two months. A trend of P^{32} retention in bone and muscle was revealed, tending in the same direction as in the nonfunctioning teeth, and a similar change in the phosphorus uptake could be produced locally in bone by resection of the muscles inserted in the region.

We do not possess data on the exchange pattern of other ions under similarly abnormal functional conditions of the teeth. Metabolism as influenced by exercise may be traced better by carbon, potassium, or sodium. In the muscle, function had entirely different effects on potassium, as compared to phosphorus, interchange.[17]

The exchange of phosphorus in the living tooth may be in part a physical phenomenon, although greatly influenced and controlled in its degree by the prevailing biologic conditions.

If, in a physical exchange concept, the size and accessibility of crystal surfaces are of consequence in exchange reactions, an increase in microporosity going hand in hand with an increased water content[18, 19] found in tissues with lessened function may possibly play a role as a physical basis for the difference in the turnover rate characteristics. However, the extent of these structural differences seems too minute to account for the large variation in the affinity to labeled phosphorus.

In attempting to explain the variance of the phosphorus turnover with function and nonfunction as a biologic phenomenon, it is conceivable to ascribe it to an active adaptive process, involving the molecular rebuilding and rearrangement of the tissue in response to abnormal functional circumstances.

SUMMARY

1. A markedly greater retention of radioactive phosphorus was found in the nonfunctioning halves of monkey dentitions as compared with functioning halves.

2. The same trend was noticed whether disuse was produced by unilateral motor nerve section, by unilateral muscle resection, or by removal of one quadrant of teeth.

3. Frequent measurements were made topically from the molar surface in the mouth of the monkey with a small end-window Geiger-Müller counter, and were repeated after extraction.

4. It appears that the exchange of phosphorus ions in the tooth depends on biologic variables and that function may manifest itself as a factor affecting the ionic balance and turnover rate.

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INCIDENCE OF DENTAL CARIES IN CHILDREN 1 TO 6 YEARS OF AGE

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INTRODUCTION

EPIDEMIOLOGICAL surveys on dental caries conducted in the last two decades have resulted in increased attention to prevention and control. However, prevention has been mostly for the permanent dentition, while preschool and kindergarten children (during the primary or mixed dentition) have received little attention for either prevention, control, or treatment (Pediatric Survey[1]). One reason for this apparent neglect is that, whereas a large number of studies on the prevalence of dental caries has been conducted in school children and adults (Klein, Palmer, and Knutson,[2] Klein and Palmer,[3] Day and Sedwick,[4] Sloman and Sharp,[5] Brekhus,[6] and others), little is known for children under 6 years of age.

In 1943, the American Association of Public Health Dentists,[7] while indicating that the measurement of caries in primary teeth is difficult because of the variations in the exfoliation time, adopted a dental examination form for recording dental caries of primary teeth in children. The system was symbolized with the lower-case letters, "def," in which "d" indicates decayed primary teeth indicated for filling, "e" indicates decayed primary teeth indicated for extraction, and "f" denotes filled primary teeth.

The relative absence of information on the incidence of caries in children under 6 years of age and the availability of a standardized epidemiological method[7] for the evaluation of dental caries prompted this investigation.

A review of the literature on prevalence of dental caries in children up to 6 years of age is summarized in Table I.[8-27] The collection of data and determination of the prevalence of any disease require certain criteria. The requirements essentially are, first, that the personnel used should be competent and well trained for determining the presence and the extent of the disease. Second, the method used for the assessment of the disease should be simple to permit examination of large samples, standardized so as to be reproducible by other investigators, and quantitative to permit easy statistical evaluation. All this should not be attained by losing accuracy in diagnosis. Third, the size as well as the selection of the sample should be determined carefully so as not to bias the findings. Fourth, the method of analyzing the data should be standardized so that comparison with other investigations in other parts of the country can be carried out easily and effectively, thereby aiding in the determination of any variable factors influencing the course of the disease.

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Variations in these criteria have resulted in accumulations of data which cannot be evaluated properly, so that only a few of the studies listed in Table I can be compared with each other.

In Table I, the first column gives the name of the author and the year in which the investigation was published. Method of examination is subdivided into the personnel conducting the examination and the instruments used. Different age groups reported are in the third column. The rest of the table gives information regarding the method of presentation of the data by the author, and the last column lists the other variable factors influencing the findings, making the study unfit for comparison with other surveys, and thereby limiting its usefulness.

MATERIALS

Six hundred fifty children, 1 to 6 years of age, living in Portland, Oregon, attending nurseries, kindergarten, and grade schools, were examined for the presence of dental caries. As the sample included the total child population available in Portland nurseries, it would constitute a relatively valid random sampling of the preschool population. Five of the nine nurseries were financed partially or completely by welfare agencies, while four were supported by tuition only. One hundred nineteen children attending public school in kindergarten and first grade were included, as the nursery school children had an age limit of 5 years.

METHODS

The examinations were carried out under good lighting conditions with No. 3 plain mirrors and fine-pointed pigtail explorers by the investigators. The alternate child went to one of the examining teams. Each team consisted of a dentist trained in epidemiological examination technic for dental caries and a dental hygienist who acted as the recorder. The def index of recording the caries experience in primary teeth, as proposed by Gruebbel,[17] was used. In addition, teeth which were undoubtedly missing because of caries were also recorded. Incidence of dental caries in the first permanent molars was recorded as DMF teeth or tooth surfaces. Teeth designated as carious were those which showed actual, although frequently small, cavities. The lesions recorded were those which were readily found on careful clinical inspection. Deep pits and fissures were not recorded as carious if they were not considered carious after thorough inspection and exploration.

An affected tooth was counted only once, that is, a tooth with a filling on the occlusal surface and decay on the mesial surface was counted as a decayed tooth, for the total def score or for the individual component d denoting decayed tooth. The same basis was used for the permanent teeth as well as for the analysis of affected surfaces.

The children were segregated into separate age groups according to their last birthday. Children of other than the white race were excluded to eliminate bias. Analysis of the data was carried out following the statistical methods of Snedecor.[28]

TABLE I

REVIEW OF LITERATURE OF DENTAL CARIES IN NORTH AMERICAN CHILDREN 1 TO 6 YEARS OF AGE

AUTHOR	METHOD OF EXAMINATION		AGE	PREVALENCE OF CARIES OR PERCENTAGE OF POPULATION AFFECTED	INCIDENCE				DMF (FIRST MOLARS)	COMMENTS
	PERSONNEL	INSTRUMENTS USED			d	e	f	def		
Techow,[8] 1916	Author	Fine explorer	6 yr.	—	—	—	—	—	—	Order of susceptibility of primary teeth given
Rypins,[9] 1922	Physicians	Tongue blade	1 to 6 yr.	Percentage	x	—	—	—	—	
Stoughton and Meaker,[10] 1931	Trained dental hygienists	Not stated	6 yr.	Percentage	—	—	—	—	% given	
Hamill and Sausser,[11] 1933	Not stated	Not stated	1 to 6 yr.	Percentage	x	—	—	—	—	All children were from low-income families
Munblatt,[12] 1933	Author	"Detailed examination"	1 to 6 yr.	Percentage	—	—	—	—	—	Children under institutional care had "better teeth" than those in private homes
McCall,[13] 1934	Dentist	Complete examination with x-ray films	2 to 5 yr.	Percentage	—	—	—	—	—	Clinical patients used whose caries incidence was very high
Cohen,[14] 1936	Not stated	Not stated	2 to 6 yr.	Percentage	% rept'd	% of missing rept'd	% rept'd	—	—	Children from clinics and institutions were used; only a small portion of the sample were public school children
Klein, Palmer, Knutson,[15] 1938	Public health dentists	Mirror and explorer	6 yr.	Percentage	x	—	x	—	x	
Ast,[16] 1944	Dental hygienist	Mouth mirror and explorer	1 to 5 yr.	Percentage	x	—	—	—	—	522 children in rural and small urban areas of New York State were examined

Gruebbel,[17] 1944	Not stated	Not stated	6 yr.	Percentage	% rept'd	% rept'd	% rept'd	% rept'd	x	
Sebelius,[18] 1944	Four dentists	Mirror and explorer	3 to 5 yr. combined	—	x	x	x	x	—	Age groups not reported individually; white and Negro children were reported separately
Chilton and Greenwald,[19] 1947	Dentists	Mirror, explorer, and x-ray films	5 and 6 yr.	—	x	—	—	x	x	Value of x-ray films as an adjunct to clinical examinations is illustrated
Finn,[20] 1947	Author	Mouth mirror and explorer	2 to 5 yr.	Percentage	x	—	x	x	x	Newburgh, Kingston study of fluoridation of water supplies
Chilton and Wisan,[21] 1948	Dentists	Mirror and explorer	4 to 6 yr.	—	x	x	x	x	x	
McLaren,[22] 1948	Author	Mirror, explorer, and magnifying loupe	5 and 6 yr.	—	—	—	—	x	—	
Dean et al.,[23] 1950	Not stated	Mouth mirror and explorer	4 to 6 yr.	—	—	—	—	x	x	Study was performed in connection with a fluoridation program
Hadjimarkos,[24] 1952	Author	Mouth mirror, explorer, and chip syringe	5 and 6 yr.	Percentage	x	x	x	x	x	Study was performed in connection with a fluoridation program; total child population of Corvallis examined; used chip syringe for drying the teeth during examination
Fulton,[25] 1952 (Illinois)	Dentists	Not stated	1 mo. to 5 yr.	—	x	x	x	x	—	Examined at Well Baby Conferences at Annual State Fair
Fulton,[25] 1952 (Cleveland)	Not stated	Not stated	5 and 6 yr.	—	x	x	x	x	—	Examined longitudinal records of 233 children from 5 years to 12 years of age
Witter,[26] 1953 (Bend)	Author	Mouth mirror and explorer	5 and 6 yr.	—	x	x	x	x	x	Prefluoridation study
Witter,[27] 1953 (Tigard)	Author	Mouth mirror and explorer	5 and 6 yr.	—	x	x	x	x	x	Prefluoridation study

FINDINGS

Fig. 1 shows the age and sex distribution of 650 children examined. The distribution of boys and girls is comparable (335 boys and 315 girls). The distribution of children is equal from 3- to 6-year age groups. There are

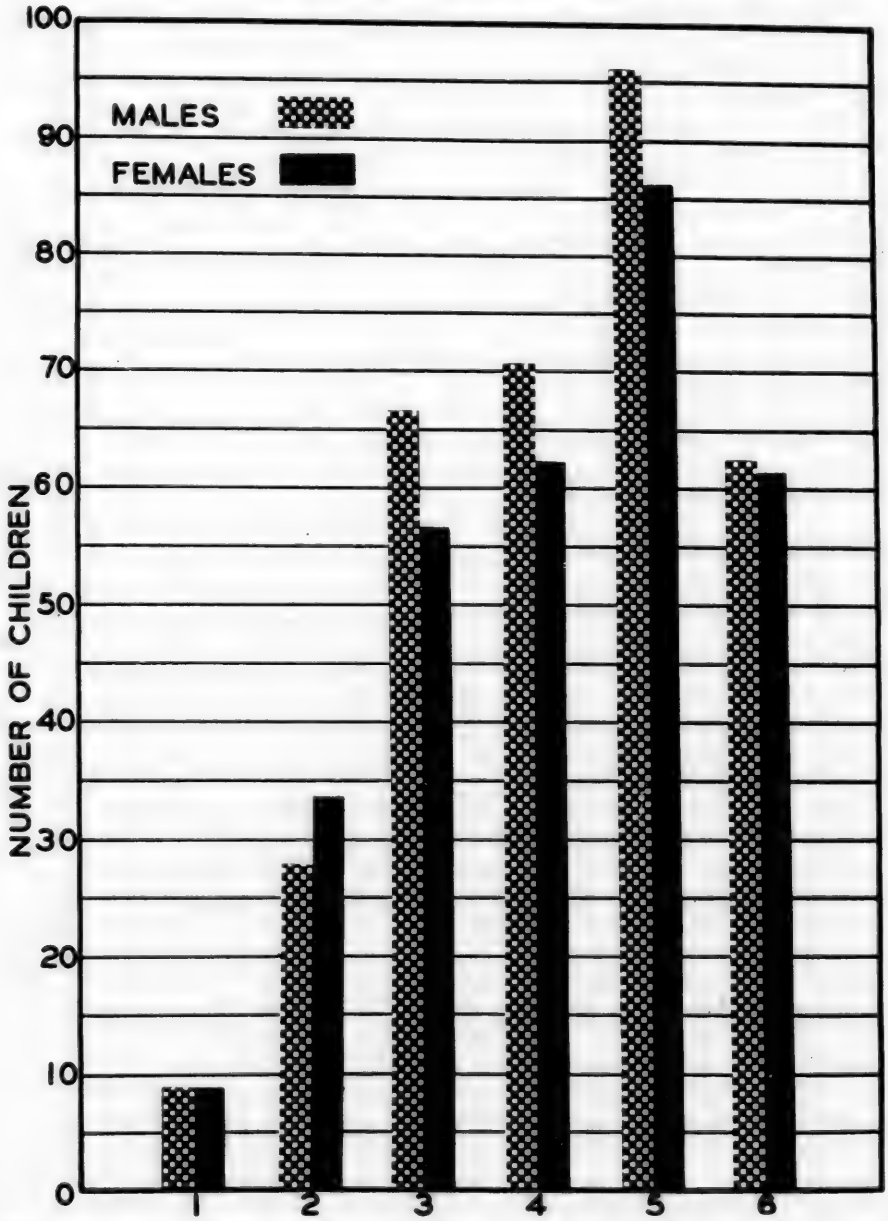

Fig. 1.—Age and sex distribution of 650 children examined in Portland (Oregon) nurseries.

eighteen 1-year-old children, while the 2-year-old age group with sixty-five children (twenty-eight boys and thirty-seven girls) may permit statistical evaluation.

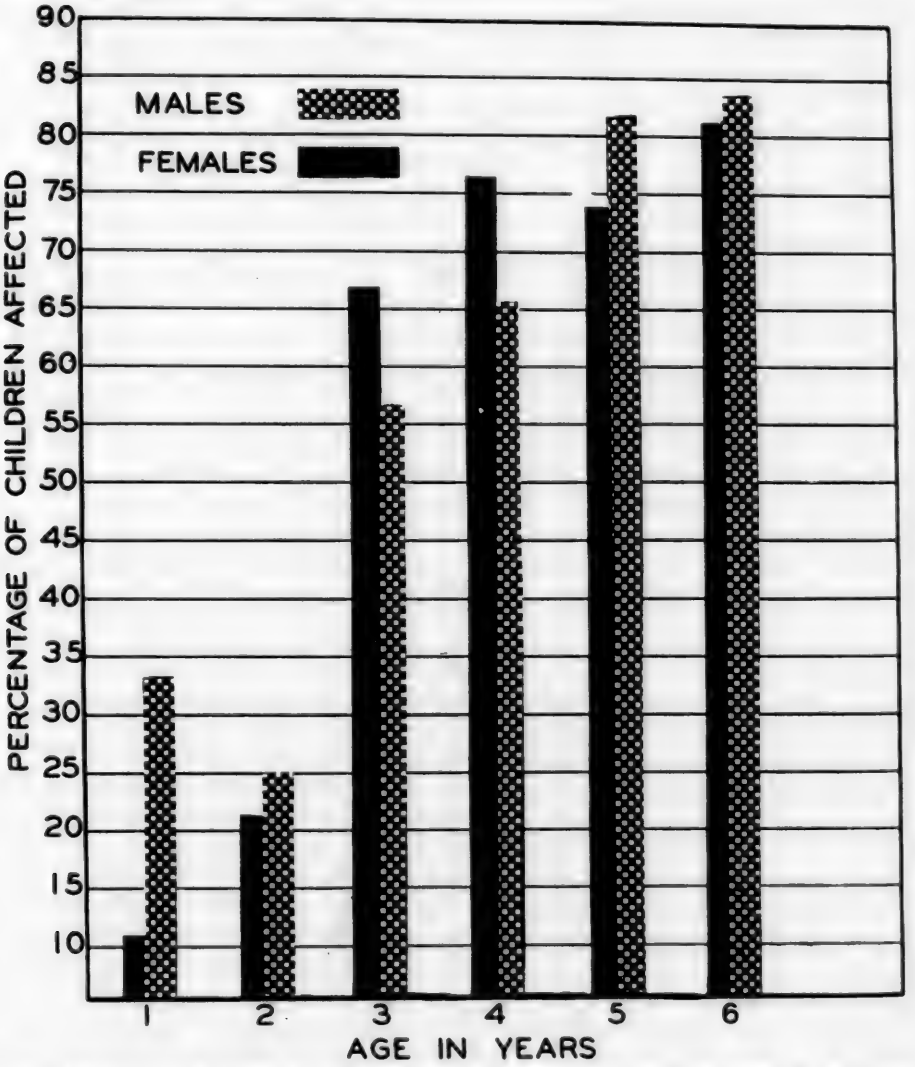

Fig. 2.—Prevalence of dental caries (percentage of children affected) in children 1 to 6 years of age attending Portland nurseries.

Prevalence of Dental Caries.—Children at 1 and 2 years of age had 22.2 per cent and 23.1 per cent decay (def index), respectively (Table II and Fig. 2). The prevalence of the disease increased rapidly to 61.8 per cent affected at 3 years, and the trend continued at a slower rate after that. At 4, 5, and 6 years, 70.9, 78.3, and 83.3 per cent of the children were affected in that order.

TABLE II

PREVALENCE OF DENTAL CARIES (PER CENT AFFECTED) IN 650 CHILDREN 1 TO 6 YEARS OF AGE

AGE (YR.)	BOYS			GIRLS			COMBINED		
	NO. OF CHILDREN	NO. WITH CARIES	% WITH CARIES	NO. OF CHILDREN	NO. WITH CARIES	% WITH CARIES	NO. OF CHILDREN	NO. WITH CARIES	% WITH CARIES
1	9	3	33.3	9	1	11.1	18	4	22.2
2	28	7	25.0	37	8	21.6	65	15	23.1
3	66	38	57.6	57	38	66.7	123	76	61.8
4	71	47	66.2	63	48	76.2	134	95	70.9
5	97	79	81.4	87	65	74.7	184	144	78.3
6	64	54	84.4	62	51	82.3	126	105	83.3
Total	335	228	68.1	315	211	67.0	650	439	67.5

The sudden increase of 23.1 per cent affected to 61.8 per cent at 3 years can be explained on the basis of the eruption of the first and second primary molars. The number of children affected increases at a slower rate at 4, 5, and 6 years because no new teeth are erupting and the number of teeth available for attack by dental caries is decreasing with increase in age.

Further analysis of these data was made according to the sex distribution of children, and it was found that the girls had a higher incidence of dental caries at 3 and 4 years as compared to boys. This difference was reversed at 5 years, but was not significant at any age level.

Dental Caries Experience.—

def teeth: The average number of def teeth per child (Table IIIA, Table IIIB, and Fig. 3*A*) was 0.67 at 1 year, 0.83 at 2 years, and rose to 2.72 def

TABLE IIIA

INCIDENCE OF DENTAL CARIES (DEF TEETH) IN 650 CHILDREN (BOYS AND GIRLS COMBINED) 1 TO 6 YEARS OF AGE

AGE (YR.)	NO. OF CHILDREN	AVERAGE def TEETH PER CHILD	STANDARD DEVIATION	MEDIAN OF def TEETH	RANGE OF DISPERSION
1	18	0.67	±1.87	0	0 to 12
2	65	0.83	±2.10	0	0 to 15
3	123	2.72	±3.05	2	0 to 19
4	134	4.05	±4.23	4	0 to 16
5	184	4.76	±4.06	4	0 to 16
6	126	5.13	±3.73	6	0 to 8

TABLE IIIB

INCIDENCE OF DENTAL CARIES (DEF TEETH AND DEF TOOTH SURFACES) FOR BOYS AND GIRLS 1 TO 6 YEARS OF AGE

AGE (YR.)	BOYS			GIRLS		
	NO. OF CHILDREN	AVERAGE NO. OF def		NO. OF CHILDREN	AVERAGE NO. OF def	
		TEETH	TOOTH SURFACES		TEETH	TOOTH SURFACES
1	9	1.11	1.5	9	0.22	0.2
2	28	1.03	1.1	37	0.68	0.6
3	66	2.71	3.9	57	2.74	3.9
4	71	3.89	7.2	63	4.22	7 3
5	97	4.74	8.9	87	4.77	9.4
6	64	4.89	10.0	62	5.37	11.3

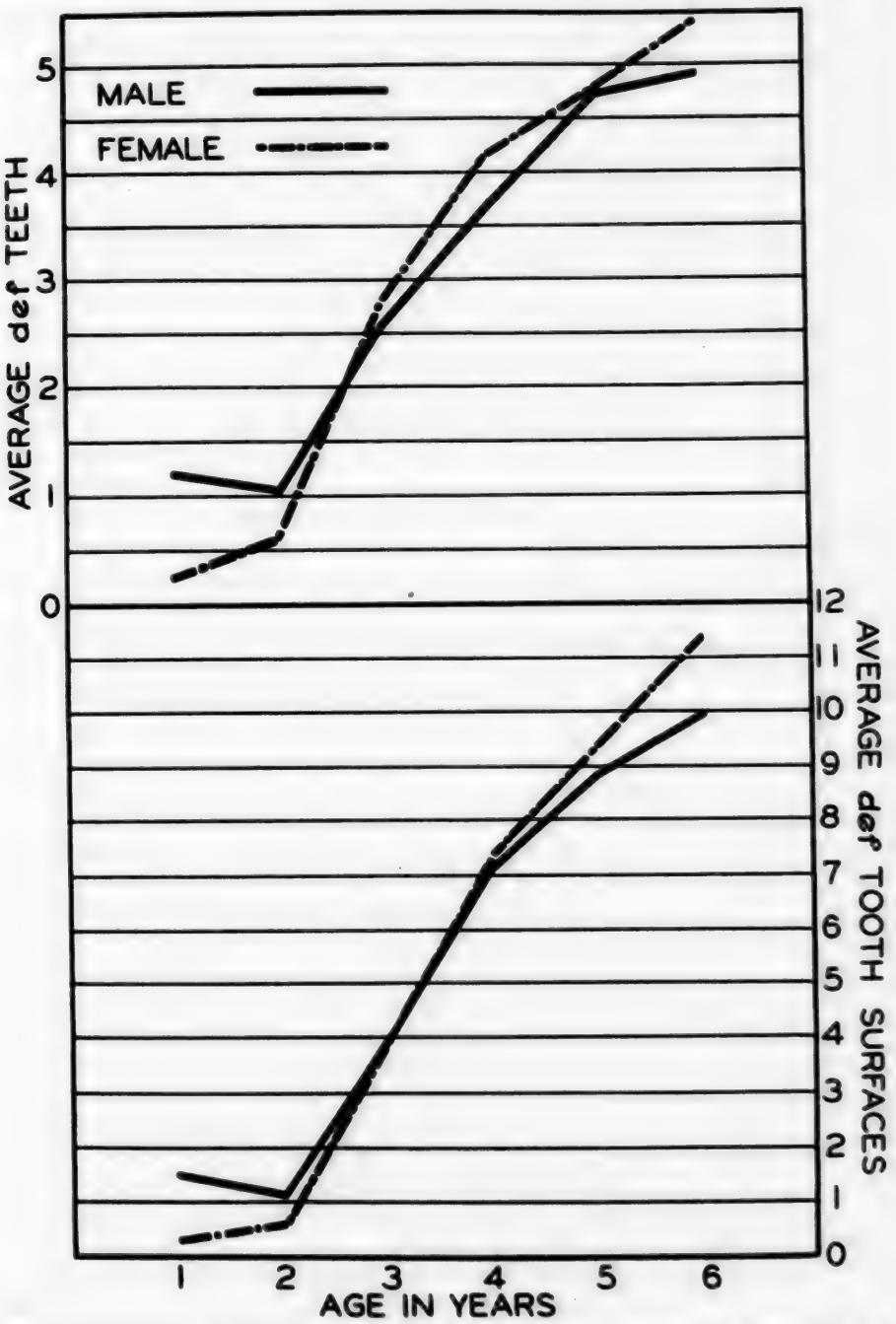

Fig. 3A.—Incidence of dental caries (def teeth and def tooth surfaces) in children 1 to 6 years of age.

teeth per child at 3 years of age. The average number of def teeth per child at 4, 5, and 6 years of age was 4.04, 4.76, and 5.13, respectively. Difference in the incidence of decay between boys and girls was insignificant.

The increase of approximately two carious teeth from 2 to 3 years of age supports the earlier contention of increase in prevalence of dental caries at 3 years of age being influenced by the eruption of the second primary molars. This indicates that the oral cavity becomes more susceptible to dental caries with the completion of eruption of the primary molar teeth, a trend which is still apparent at 4 years of age. The decreased involvement of teeth by caries at 5 and 6 years of age is due to the decreased number of ssuceptible teeth available for caries attack.

Dental caries is an accumulative disease, and its prevalence increases with age. A more accurate statistical interpretation of data of this disease in its incipient stages is illustrated in Fig. 3*B*, which shows the percentile distribution of children according to the number of def teeth. The data are illustrated at three class intervals, that is, 1 and 2 years, 3 and 4 years, 5 and 6 years, thus reducing the number of lines on the graph and emphasizing the salient features.

It is inaccurate to use the term "average amount of decay" at certain age levels, as a relatively small portion of the population is affected, and the term "average" does not discriminate and point out such variations in affected populations. For example, at 1 and 2 years, 79 per cent of the children had no decay (Fig. 3*B*); only 33 per cent of the 3- and 4-year-old children had no decay; and only 19 per cent of the 5- and 6-year-old children were caries free. To illustrate further, only 1 per cent of the 2-year-old children showed as many as six carious teeth, whereas 16 per cent of the 5- and 6-year-old children had this many affected teeth. Taking this into consideration, the standard deviation, the median, and the range of dispersion of carious teeth (def) for each age group were computed and are shown in columns three, four, and five of Table IIIA. These figures aid in proper interpretation of the true incidence of caries in its earlier stages. For example, at 2 years of age, the average child had 0.83 affected teeth, whereas the median value was 0, showing that the central tendency for that age group was to have no caries. This indicates that less than 50 per cent of the children were affected (as seen in Table II, only fifteen of sixty-five children had carious teeth). The range of dispersion for these 2-year-old children is 0 to 12.

def tooth surfaces: Incidence of the average number of def tooth surfaces involved is seen in Table IIIB and Fig. 3*A*. In general, the average girl showed a lower incidence of dental caries before 3 years as compared to the boy, but this incidence was reversed after 3 years of age, and the difference was not significant at any age level.

The average boy had one surface involved at 1 and 2 years, and 3.9 surfaces at 3 years. The incidence doubled to 7.2 surfaces at 4 years, and remained steady at 5 and 6 years, the involvement being 8.9 and 10.0 def tooth surfaces per child.

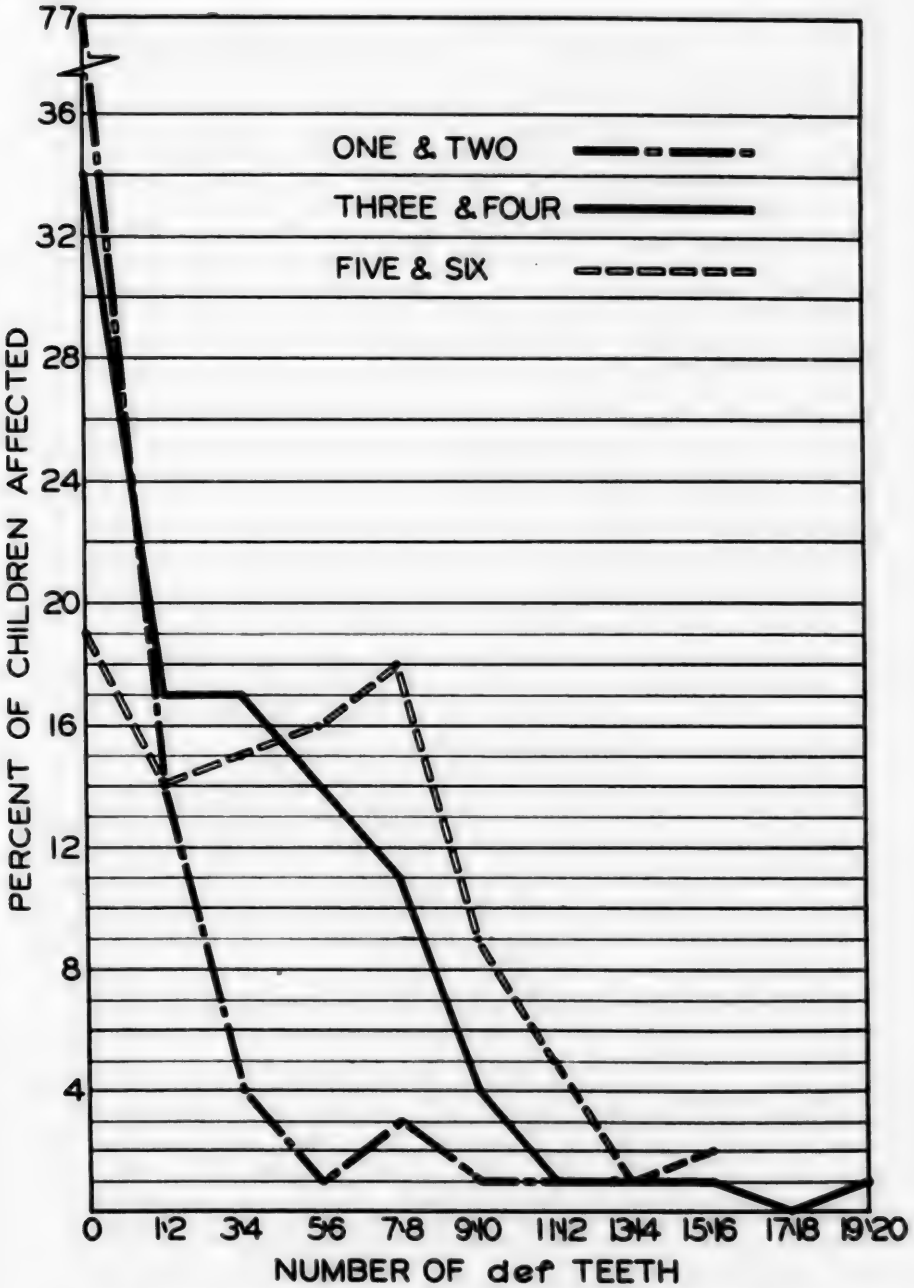

Fig. 3*B*.—Severity of dental caries (def teeth) in children 1 to 6 years of age.

Girls showed less than one surface involved at 1 and 2 years, 3.9 surfaces affected at 3 years, 7.3 surfaces at 4 years. The involvement of surfaces in girls was higher than boys at 5 and 6 years, being 9.4 and 11.3, respectively. The ratio betwen teeth and def tooth surfaces was 1:1 at 1 and 2 years, 1:1.5 at 3 years, and increased to 1:2 at 4, 5, and 6 years.

Analysis of d, e, and f Teeth.—Table IIIC shows that 0.67 decayed tooth (teeth requiring restorations) per child was present at 1 and 2 years of age. The figures were 2.63 and 3.45 at 3 and 4 years, respectively, and 3.64 and 3.40 at 5 and 6 years of age, respectively. The average number of fillings present in each child's mouth increases from 0.09 at 3 years to 0.45 at 4 years and to 1.6 at 6 years. As stated earlier, some teeth which had caries as well as fillings were counted as decayed teeth and are therefore not included in these figures of filled teeth.

Column 5 of Table IIIC gives the average number of teeth "indicated for extraction." Fig. 3*C* shows the same incidence graphically in comparison with the decayed, extraction indicated, and filled teeth. Maximum teeth indicated for extraction is 0.13 tooth per child or thirteen teeth per 2,000 teeth (or per 100 children) at 6 years of age. It should be clarified here that the average child who has teeth with extensive decay, that is, indicated for extraction, would tend to have more than one tooth in this category because of the bilaterality and symmetrical distribution of dental caries. The number of teeth that are recommended for extraction because of caries therefore appears to be insignificant in relation to the total caries incidence for these children at any age level.

TABLE IIIC

AVERAGE NUMBER OF def TEETH (DECAYED, EXTRACTION INDICATED, AND FILLED) FOR CHILDREN 1 TO 6 YEARS OF AGE

AGE (YR.)	NO. OF CHILDREN	AVERAGE NO. def TEETH PER CHILD	AVERAGE NO. DECAYED (d) TEETH PER CHILD	AVERAGE NO. EXTRACTIONS (e) INDICATED PER CHILD	AVERAGE NO. FILLED (f) TEETH PER CHILD
1	18	0.67	0.67	0.00	0.00
2	65	0.83	0.67	0.00	0.00
3	123	2.72	2.63	0.00	0.09
4	134	4.05	3.54	0.06	0.45
5	184	4.76	3.64	0.03	1.09
6	126	5.13	3.40	0.13	1.60

Dental Caries in the First Permanent Molar.—Table IV presents the data of carious first permanent molars at 5 and 6 years of age. One or more decayed first permanent molars were present in 29.7 per cent of children at 5

TABLE IV

PREVALENCE OF DENTAL CARIES IN THE FIRST PERMANENT MOLARS AT 5 AND 6 YEARS OF AGE

AGE (YR.)	NO. OF CHILDREN WITH 1 OR MORE MOLARS ERUPTED	CHILDREN WITH AFFECTED MOLARS		TOTAL NO. OF FIRST MOLARS AVAILABLE	AFFECTED MOLARS	
		NO.	%		NO.	%
5	37	11	29.7	93	24	25.8
6	80	37	46.3	265	106	40.0

years of age and in 46.3 per cent of children at 6 years of age. Table V further shows that 25.8 per cent of the first molars were decayed at 5 years, and the incidence rose to 40.0 per cent at 6 years.

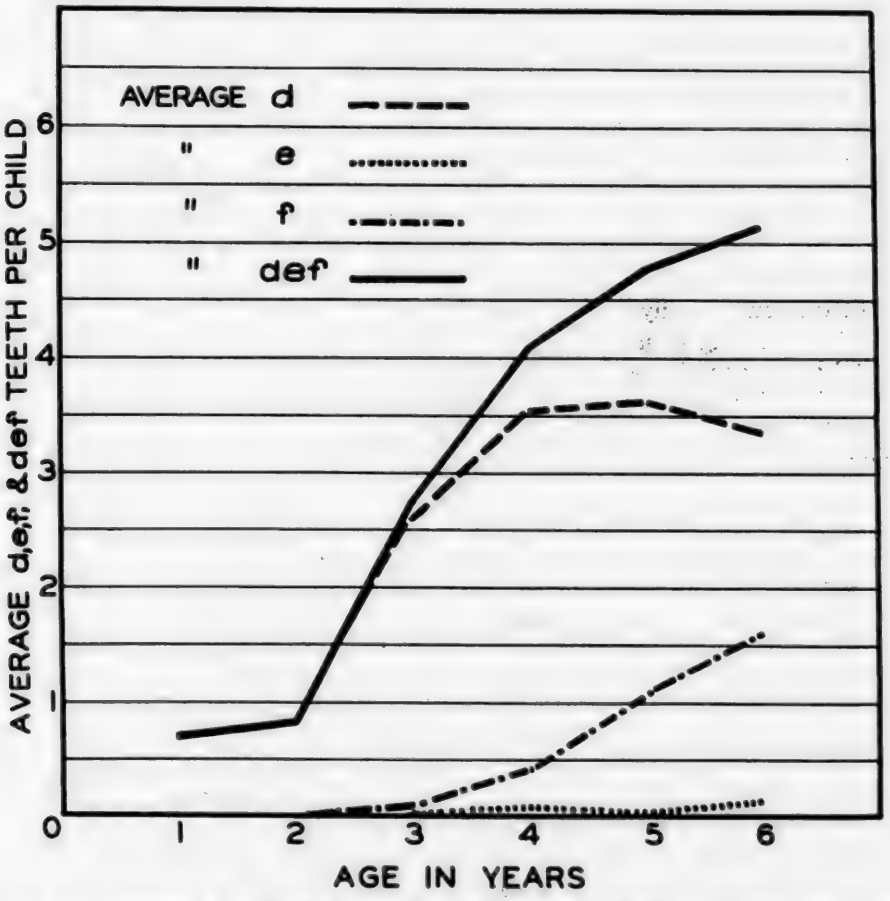

Fig. 3C.—Incidence of d (decayed teeth), e (indicated for extraction), f (filled teeth), and total def teeth in children 1 to 6 years of age.

Table V presents the compilation of the incidence of dental caries at 5 and 6 years of age as a combined value of def teeth and DMF teeth. It was felt that the combined values would give a more complete picture of the status of dental caries in the average child's mouth at these ages than either one of these indices. The combined score is 4.89 def and DMF teeth at 5 years and 5.97 at 6 years of age.

A closer examination reveals that the major portion of the increase is provided by the DMF rate (the first permanent molar), whereas the rate of involvement of the primary teeth shows a tendency to decrease after 5 years of age.

TABLE V

COMBINED INCIDENCE OF def TEETH AND DMF TEETH OF CHILDREN AGED 5 AND 6 YEARS

NO. OF CHILDREN	AGE (YR.)	def TEETH	DMF TEETH	COMBINED SCORE
184	5	4.76	0.13	4.89
126	6	5.13	0.84	5.97

Comparison of Missing Primary Teeth and Teeth Indicated for Extraction.—It was felt that the validity of the def index should be tested for children 1 to 6 years of age, as the teeth which have been lost through caries are not included and, perhaps, may constitute an important part of a child's total caries experience, and the primary teeth exfoliated up to 6 years of age (incisors) can be determined and eliminated. Table VI indicates that the maximum number of missing teeth was 0.12 per child at 6 years. This number is small and could readily be due to human error.

Thus, scoring of missing primary teeth in addition to the total count of dental caries experience is of little value in children under 7 years of age.

Table VI also compares the incidence of e to M teeth. The incidence in both the items is negligible, and if not recorded would not have biased the findings in this investigation.

TABLE VI

INCIDENCE OF PRIMARY TEETH INDICATED FOR EXTRACTION AND MISSING TEETH IN 650 CHILDREN 1 TO 6 YEARS OF AGE

AGE (YR.)	NO. OF CHILDREN	MISSING TEETH		TEETH INDICATED FOR EXTRACTION	
		TOTAL	AVERAGE PER CHILD	TOTAL	AVERAGE PER CHILD
1	18	0	00.0	0	00.0
2	65	0	00.0	0	00.0
3	118	2	00.02	0	00.0
4	134	3	00.02	8	00.06
5	184	17	00.09	5	00.03
6	126	15	00.12	17	00.13

DISCUSSION

Brekhus[29] found that dental caries experience showed a definite and significant increase every decade in freshman students in Minnesota. It was felt that a comparison of the present investigation (data were collected during winter 1950-1951) with other investigations carried out more than ten years ago would not yield any conclusive findings. The comparison of the present study, therefore, was made with children from three other towns in Oregon, six groups of children in other parts of the United States, and one group of children from Canada (Table VII).

Incidence for Portland, Bend, and Tigard children was very similar at 6 years of age, while that of Corvallis children (Hadjimarkos) was 0.5 def tooth higher. This difference between Corvallis and Portland children could be attributed to consistent use of chip syringe for drying the teeth during examination.

TABLE VII

COMPARISON OF INCIDENCE OF DENTAL CARIES (DEF TEETH) IN CHILDREN OF 1 TO 6 YEARS BETWEEN THE PRESENT AND TWELVE OTHER STUDIES

AUTHOR	1	2	3	4	5	6	COMMENTS
Savara and Suher (present study) (1951)	0.67	0.83	2.72	4.05	4.76	5.13	
Witter[26] (Bend, Ore.) (1953)	–	–	–	–	–	5.3	Prefluoridation study
Witter[27] (Tigard, Ore.) (1952)	–	–	–	–	–	5.26	
Hadjimarkos[24] (Corvallis, Ore.) (1952)	–	–	–	–	4.48	5.67	Prefluoridation study
Fulton[25] (Illinois) (1941)	0.02	0.22	1.06	2.82	4.07	–	
(Cleveland, Ohio)	–	–	–	–	5.38	6.19	
Dean, Arnold, Jay, and Knutson[23]							Fluoridation study
(Grand Rapids) (1944-45)	–	–	–	4.2	5.4	6.4	
(1949-50)	–	–	–	2.7	3.3	4.6	
(Aurora) (1945-46)	–	–	–	2.1	2.8	3.4	
(Muskegon) (1944-45)	–	–	–	–	6.8	7.2	
(1949-50)	–	–	–	4.4	5.6	6.0	
Wisan and Chilton[21] (1948)	–	–	–	3.10	3.62	4.24	
McLaren[22] (1948)	–	–	–	–	3.4	4.5	Computed

Dental caries experience at 1, 2, and 3 years of age is higher in children at Portland, Oregon, as compared to that of Illinois children reported by Fulton:[25] The difference in incidence between the two groups increases at 4 years and decreases at 5 years. It is interesting to observe that the incidence of caries at 4 years in Portland children is equal to the incidence in Illinois children at 5 years of age. The yearly increase is maximum at 3 and 4 years of age in the Portland children whereas it is maximum at 4 and 5 years in Illinois children.

Fulton's analysis of the data of Cleveland, Ohio, children revealed a higher incidence of caries at 5 and 6 years of age, the difference between the averages at 6 years of age being 1.06 def teeth. It appears that Cleveland children have a higher rate of decay as compared to Portland children and the accelerated rate tends to continue for a longer period, whereas caries rate in Portland children slowed down after 5 years of age.

Incidence of dental caries of children at Grand Rapids, Michigan, was higher in 1944-1945 than that of Portland children, whereas the figures obtained after five years of fluoridation (1949-1950) are lower. However, Aurora children showed still a lower incidence. Muskegon children used as a control group had higher incidence than Portland children in 1944-1945 and 1949-1950. Figures for 1949-1950 Muskegon children indicate a decline in the rate of involvement after 5 years, similar to the one reported for Portland children at 4 years. Chilton and Wisan's[21] report indicates a similar trend of a large increase at 5 years as compared to 4-year-old children, with an incidence of 3.10 at 4 years indicating a fairly high caries attack rate during 2 and 3 years of age. McLaren[22] reported a similar incidence at 5 years for Toronto children.

Columns 2 and 3 of Table VIII compare the incidence of first permanent molars at 5 and 6 years of age in various studies. Incidence was low at Bend, Oregon (Witter[26]). Grand Rapids children, 1949-1950, after fluoridation showed a low incidence equal to Aurora. Wisan and Chilton[21] reported a high incidence of 1,054 DMF teeth for their 6-year-old children. In children 5 years of age, McLaren[22] reported a high incidence which compared favorably with 5-year-old children examined by Wisan and Chilton and 6-year-old children reported by most of the investigators.

TABLE VIII

COMPARISON OF INCIDENCE OF DENTAL CARIES OF FIRST PERMANENT MOLAR AND COMBINED def AND DMF TEETH IN 5- AND 6-YEAR-OLD CHILDREN

AUTHOR	DMF TEETH OF FIRST PERMANENT MOLARS		COMBINED def AND DMF SCORES		COMMENTS
	5 YR.	6 YR.	5 YR.	6 YR.	
Savara and Suher (present study) (1951)	0.13	0.84	4.89	5.97	
Witter[26] (Bend, Ore.) (1953)	–	0.38	–	5.68	Prefluoridation study
Witter[27] (Tigard, Ore.) (1952)	–	0.77	–	6.03	
Hadjimarkos[24] (Corvallis, Ore.) (1952)	–	0.75	4.48	6.42	Prefluoridation study
Dean, Arnold, Jay, and Knutson[23]					
(Grand Rapids) (1944-45)	0.11	0.78	5.51	7.18	
(1949-50)	0.03	0.38	3.33	4.98	
(Aurora) (1945-46)	0.06	0.28	2.86	3.68	
(Muskegon) (1944-45)	0.06	0.81	6.86	8.01	
(1949-50)	0.14	0.63	5.74	6.63	
Wisan and Chilton[21] (1948)	0.64	1.54	4.16	5.78	
Chilton and Greenwald[19] (1947)	–	0.73	3.33	4.38	
McLaren[22] (1948)	0.45	0.70	3.85	5.20	Computed

Columns 4 and 5 of Table VIII show the comparison of incidence of total dental caries (def and DMF teeth) for children in different studies. Incidence of caries of Bend children is slightly low, while that of Corvallis children is slightly high, yet both could be a statistical error. A higher incidence was reported for Grand Rapids children (1944-1945), whereas a lower incidence was reported in postfluoridation children at Grand Rapids (1949-1950) and Aurora. McLaren[22] showed the Toronto children to have a lower incidence, whereas Chilton and Greenwald[19] found the experience to be less in New Jersey children.

SUMMARY

This investigation is based on dental caries examination of 650 children, 1 to 6 years of age, attending day nurseries, kindergarten, and grade school, and reports the prevalence and incidence of dental caries of primary teeth in those children. Incidence of caries in the first permanent molar in 5- and 6-year-old children is also presented. No significant sex difference was found. The number of primary teeth missing (extracted because of dental caries) as well as teeth indicated for extraction was very small. Findings of this study were compared to other studies reported in the last ten years in North America.

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LOCAL AND SYSTEMIC CARIOGENIC EFFECTS OF REFINED DEXTROSE SOLUTION FED TO ONE ANIMAL IN PARABIOSIS

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THE clinical impression of the deleterious effects of a highly refined carbohydrate diet on human teeth has been strengthened by the findings of Koehne and Bunting,[1] Boyd,[2] and others. Animal experimentation by Shibata,[3] McClure,[4] Keyes,[5] Elvehjem,[6] and Sognnaes[7] adduced similar findings. Lundquist's[8] study of oral sugar clearance indicates that deproteinized fasting saliva from normal individuals is sugar free and that hyperglycemia, whether pathologic or due to high consumption of sugar, does not entail any transfer of sugar to the saliva. Furthermore, he stated that the saliva becomes sugar containing only in so far as carbohydrates are secondarily dissolved in it, and that a high sugar concentration in the saliva, in conjunction with prolonged clearance time, entails increased caries activity.

At the present time, investigations concerning the etiology of dental caries suggest that initiation of dental caries may be an acidogenic or a proteolytic process. The large number of individual variations inherent in both animal and human experimental material has impeded our understanding of the dental caries processes. Previous reports by Kamrin,[9] Finerty,[10] and Gumbreck[11] have indicated that successfully parabiosed albino rats can be considered to be genetically similar, and suggest their use as a method of reducing individual variation. Thus, in properly planned experiments, one of the parabionts (surgically united co-twin) may be utilized as the experimental animal while the other co-twin serves as a control.

Shibata's[3] dextrose feeding experiments showed that this refined carbohydrate had cariogenic potency, although Kesel[12] was unable to confirm his results. However, in previous work,[9] in which a high concentration of dextrose was fed to single and parabiosed animals, it was found that the addition of refined carbohydrate was instrumental in initiating dental caries. It was significant that successfully parabiosed albino rats showed a similar pattern and incidence of dental caries in both parabionts. This was not the case in unsuccessful parabiotic animals, or in un-united littermate pairs. There was no clear correlation between the age or weight of the animals and the incidence of dental caries. However, it was established that refined dextrose mixed in equal parts with a balanced diet will result in discernible dental caries by the forty-sixth day of age (twenty-five days of feeding time) in the mandibular molar teeth and by the sixty-first day of age (forty days of feeding time) in the maxillary teeth.

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The present work concerns experiments planned to determine the local, systemic, or combined role of a refined monosaccharide (dextrose) in the initiation of dental caries. It is our belief that in successfully parabiosed animals (those having genetic similarity, common circulation, equal metabolic processes, and equal environment), individual variation is markedly reduced or eliminated. Therefore, by feeding a dextrose solution to one of the parabionts, both local and systemic effects of the diet could be studied, while in opposite parabionts only systemic effects would be operative.

METHODS AND MATERIALS

Fifty-five pairs of littermates (Wistar albino rats) were joined in parabiosis; of these, forty-one pairs survived for a period longer than fifteen days. Of the survivors, thirteen pairs were maintained on a ground balanced diet* and were sacrificed at periods varying from sixteen to 170 days. The remaining twenty-eight experimental-control parabiotic pairs were maintained in the same environment and fed on the same balanced diet as the control groups. In the experimental group, the right member of the parabiotic pair was fed 1.0 c.c. of 25 per cent dextrose solution daily, and sacrificed after receiving the dextrose solution for sixteen to 105 days.

The initial figure of sixteen days was chosen to eliminate the unsuccessful parabiotic pairs in which one parabiont remains stunted and complicates the problem by adding the variations of debility and autointoxication. From previous investigations, it was found practical to separate all parabiotic pairs into:

A. *Unsuccessful parabiosis:* Class 1: stunting and death of one parabiont within fifteen days; Class 2: equal growth of both parabionts with constant separation of the suture line and tearing apart of the animals, alopecia near operated area, and poor healing of wounds.

B. *Successful parabiosis:* Equal growth of parabionts, healthy union of suture lines with complete elimination of scar by thirtieth day, and no alopecia. Therefore, it will be noted that all nonseparating parabiotic animals surviving past the thirty-first day will be successful and can be maintained for long periods of time.

At sacrifice of the animals, both the mandible and maxilla were excised, the soft tissues removed, and the jaws examined under the dissecting microscope. Notations were made at this time of the dental findings as ascertained by a fine-pointed probe. The visualization was facilitated by clearing the jaws in aniline, according to the method suggested by Leicester and Schamp.[13] After six to ten days of clearing, the jaws were again examined under the microscope for any carious lesions missed in the first examination.

Charting was done on the following basis:

III	II	I	I	II	III
III	II	I	I	II	III

*Food pellets manufactured by Cooperative GLF Marketing Service Inc., Canandaigua, New York. It consists of natural food products containing 44 per cent carbohydrates, 29 per cent crude protein, 8.28 per cent crude fat, and 1.58 per cent salt. It is additionally fortified with vitamins and minerals. These pellets were ground so that 75 per cent of the particles passed through a 30-mesh screen.

Each of the dots adjacent to a Roman numeral indicates the number of fissures normally present in that tooth (molar). A rat showing carious processes in all the available fissures would have an incidence of twenty-two fissure caries or 100 per cent decay. This procedure was deemed feasible because no other area of the molar crown was ever involved in caries during the use of dry or liquid dextrose.

Determination of glucose levels in the blood were by the Somogyi[14] method. Blood was drawn from the jugular confluence in the neck at one-half-hour intervals.

RESULTS

The control group, which consisted of thirteen pairs of parabiotic albino rats maintained for a period varying from sixteen to 150 days on a balanced diet (no refined sugar), was free of dental caries, with the exception of the oldest pair. This pair in parabiosis for 150 days (age 171 days) developed one molar carious cavity in each rat.

Although the growth curves of both the experimental and control groups of parabiotic animals are similar, the incidence of dental caries is markedly different in the experimental group. Table I indicates the incidence of dental caries induced by the experimental procedures. It is significant that the greatest incidence of fissure caries occurs in the shortest space of time when the monosaccharide feeding is initiated immediately after weaning. It is also significant that the left parabiont, which received its glucose through the systemic circulation from the right glucose-fed parabiont, had a mean caries rate of 0.17, as compared with 2.43 for the experimental animals.

TABLE I

INCIDENCE OF DENTAL CARIES IN PARABIOSIS BY DEXTROSE SOLUTION (ONLY RIGHT PARABIONT FED 1.0 C.C. OF 25 PER CENT DEXTROSE SOLUTION)

AGE OF PARABIONTS AT BEGINNING OF DEXTROSE FEEDING (DAYS)	DURATION OF DEXTROSE FEEDING (DAYS)	NUMBER OF PAIRS	TOTAL POSSIBLE NO. OF FISSURES IN GROUP	LEFT PARABIONT			RIGHT PARABIONT		
				TOTAL F.C.*	% OF F.C.*	MEAN	TOTAL F.C.*	% OF F.C.*	MEAN
				Group A					
21-24	16-35	8	176	4	2.2	0.5	29	16.5	3.62
25-28	16-35	4	88	0	0.0	0.0	12	13.6	3.00
Over 29	16-35	6	132	0	0.0	0.0	4	3.0	0.66
			Average	4 total		0.17	45 total		2.43†
				Group B					
21-24	36-105	3	66	2	4.5	0.66	15	22.7	5.00
25-28	36-105	2	44	0	0.0	0.0	6	13.6	3.00
Over 29	36-105	5	110	7	6.4	1.4	35	31.8	7.00
			Average	9 total		0.69	56 total		5.00†

*f. c. = fissure caries.
†Significant at 0.01 per cent level by "t" test.

In the older age group of experimental animals, those over 29 days of age when glucose feeding was initiated and continued for periods up to 105 days, the mean caries formation was 7.0 for the right parabiont and 1.40 for the left

control parabiont. This value was somewhat higher than that found in the 21-to-24-day age group, which was maintained on the dextrose for the same period of time. However, combination of the 21-to-24-day age groups of both A and B of Table I (feeding for 16-35 days and 36-105 days) clearly indicates the preponderance of caries when dextrose feeding is started immediately after weaning.

Analysis of the blood glucose by the Somogyi[14] method demonstrated that there is no significant difference in the amount of blood glucose between the right and left parabionts despite the initial feeding of the right parabiont with 250 mg. of dextrose. Equalization of glucose concentration appears to occur within one hour after ingestion. This would further substantiate the belief that blood circulation is sufficient in parabiosis to assure this end.

DISCUSSION

Individual variation is one of the major obstacles in ascertaining the etiology of dental caries. Previous experiments[9] on parabiotic littermates strongly suggest that individual variation is diminished or eliminated between the successfully paired parabionts. This view is strengthened by the finding that successfully parabiosed rats which are fed on a 50 per cent dry dextrose and balanced diet will form the same pattern and incidence of dental caries in the molars.

Another factor which requires clarification in the etiology of dental caries, in so far as the role of refined carbohydrates is concerned, is whether the action is local, systemic, or a combination of both. The present experiment was devised to obtain an answer to this problem. With the reduction in individual variation, and the application of the refined dextrose to the oral cavity and gastrointestinal tract of only one parabiont, it can be safely assumed that the effect of such application will be both local and systemic in that parabiont. Huff, Trautman, and Van Dyke[15] and Van Dyke, Huff, and Evans[16] have definitely established that there is capillary blood interchange between the surgically united pairs equal to ten or more times the blood volume of each rat, that is, 50 to 150 c.c. each twenty-four hours. The similarity of the glucose blood level in each member of our parabiotic pairs also indicates uniform blood distribution. From this it may be assumed that any effect caused by the presence of glucose will be purely systemic. The experimental work of Lundquist[8] on human beings clearly demonstrated that hyperglycemia does not entail any transfer of sugar to the saliva. If this is true for the rat, it also indicates that any action of dextrose on the left parabiont in the initiation or facilitation of dental caries is solely a systemic action.

Tabulation of the experimental data, which are statistically significant at the 0.01 per cent level, demonstrates that the effect of feeding 1 c.c. of 25 per cent dextrose solution to one parabiont in the development of carious dental lesions is predominantly, if not solely, local in action. Cariogenicity is most evident when dextrose feeding is begun immediately after weaning, such carious

lesions being demonstrated as early as the sixteenth day of sugar feeding. Prolonged feeding of dextrose to older parabionts will also result in the decay of the molar fissures. Although the incidence is most marked in the mandibular molars, the maxillary molars are not immune. This finding is of interest, because many of the investigators in this field believe that either a coarse ration or a sticky one is necessary to initiate caries. This ration was neither coarse nor sticky, as little food was found impacted in the fissures except where caries were found. No cavities were found in the incisors.

The finding of dental caries in the nondextrose-fed left parabiont (Group A: Mean—0.17 caries per rat, and Group B: Mean—0.6 caries per rat) may indicate that other factors should be considered in the etiology of dental caries. Successful parabiosis, itself, is not a debilitating factor, for the surgically united animals had as consistent a gain in weight as the un-united animals. A simple explanation for the caries phenomena in the left parabiont may lie in the observation that there is much licking of the opposite faces by the rats, so that any spilled or regurgitated dextrose may be ingested by the untreated animal. If this consideration be true, the etiology of dental caries as initiated by monosaccharides may be considered to be wholly local in action, aided by the presence of acidogenic bacteria, as demonstrated by Klein and Harris,[17] Parsons, McCollum, and Frobisher,[18] and others.

CONCLUSION

1. Dextrose fed to the right parabiont of united genetically similar pairs results in a high incidence of dental decay (local and systemic effects) and a negative or minimal cariogenic effect on the left parabiont (systemic effect only).

2. Statistical analysis of the increased incidence of caries induced by the high carbohydrate diet was highly significant by the t test.

3. The addition of dextrose solution to the normal balanced diet does not appear to have any effect upon the normal growth of the animals.

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DENTAL CARIES IN THE SYRIAN HAMSTER

VI. Minimal Dental Caries Activity in Animals Fed Presumably Cariogenic Rations

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IN PREVIOUS studies, Syrian hamsters have been found highly susceptible to dental caries when maintained under average laboratory conditions, bred and raised on commercially prepared diets with fresh vegetable supplements, and fed several types of nonpurified cariogenic rations.[1-3] Other workers have occasionally experienced difficulty in reproducing these findings, and there has always been considerable variation in caries susceptibility between animals, including littermates.

This study was undertaken as an attempt to obtain a somewhat more uniform incidence of dental caries in Syrian hamsters by (1) careful control of breeding, (2) maintaining uniform laboratory conditions, (3) use of purified or semipurified diets, and (4) precise control of the experimental period, from twenty-one to 121 days.

PROCEDURE

In February, 1950, three female and three male hamsters were obtained for breeding purposes from the hamster colony at the University of Rochester. This colony was selected because animals from this group have shown rather consistent susceptibility to dental caries on a wide variety of rations and under variable laboratory conditions.

However, only one of the females became pregnant, and it produced two litters of three animals each (four females and two males). These animals were inbred by brother-sister matings until sufficient animals were available for experimental studies.

The breeding procedure has been to keep females in screen-bottomed cages, to check daily the stage of the estrus cycle by methods described by Ward,[4] and to place a male in the cage with the female the evening of estrus. The male is removed the next morning, and the date of mating is recorded. The female is observed during the following ten to twelve days to check on the development of pregnancy. If pregnant, the female is transferred to a larger cage with shavings for nesting material several days before animals are to be born. After weaning, at 21 days, the young animals are transferred to screen-bottomed cages in conventional racks. Detailed records are kept of each female

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breeder: for example, estrus cycle, number of male and day of mating, date of delivery, approximate number of animals born, number of animals weaned, and so forth.

All animals have been bred, raised, and kept in animal quarters in which uniform temperature of 80° F. is maintained by carefully regulated air conditioning. Incandescent light, which is the only source of illumination, is controlled automatically to a fourteen-hour light cycle year round. As a result, no difficulty is experienced with breeding at any time of the year.

Diets have been of several types. When reproduction has been poor, mortality of litters high, or when test diets have not induced desired results, it has been necessary to shift from one ration to another. Also, as studies proceeded and results became available, some animals were shifted to other dietary regimens before fully completing the test period initially planned. The diets used are as follows:

Purina Laboratory Chow (PLC).

Purina Rabbit Chow Checkers (PRC).

Ration 100 (R100): Described by Shaw.[6] Sucrose 67 per cent, casein (devitaminized) 24 per cent, salts 4 per cent, corn oil 5 per cent. Each 100 Gm. of ration contains thiamine HCl 350 μg, riboflavin 350 μg, pyridoxine HCl 350 μg, nicotinic acid 2.5 mg., calcium pantothenate 2.0 mg., choline chloride 100 mg., inositol 100 mg., para-amino benzoic acid 30 mg., beta-carotene 1.1 mg., irradiated ergosterol 300 I.U., 2 methyl-1,4-naphthoquinone 0.6 mg., and alpha-tocopherol 5.0 mg. To each 100 Gm. of this diet was added 2.0 Gm. of 1:20 liver concentrate powder and 2.0 Gm. of whole liver substance. The salt mixture was that of Phillips and Hart[7] as modified by Shaw.[6]

Ration 1 (R1): Sucrose 60 per cent, casein (devitaminized) 21 per cent, corn oil 5 per cent, liver concentrate 1:20 5 per cent, whole powdered milk 5 per cent, modified salts 4 per cent,[6] celluflour 8 Gm. per 100 Gm. Each 100 Gm. of diet contained added vitamin supplements in amounts similar to those reported for Ration 100.

Ration 2 (R2): This diet is identical to Ration 1 except that 4 per cent Purina Laboratory Chow ash was substituted for the 4 per cent modified salt mixture.[6] The ash was prepared by ashing Purina Laboratory Chow at 1,050° F. for twenty-four hours.

Ration 4 (R4): Whole wheat flour 30 per cent, whole powdered milk 30 per cent, confectioners' sugar 15 per cent, cornstarch 20 per cent, alfalfa meal 4 per cent, NaCl 1 per cent.

Ration 4a (R4a): Whole wheat flour 30 per cent, sucrose 35 per cent, whole powdered milk 30 per cent, alfalfa meal 4 per cent, NaCl 1 per cent.

Food was supplied ad libitum in ceramic cups. Water was provided by means of inverted glass bottles and tubes with rubber stoppers. Fresh carrots were supplied from the seventh to twenty-first days of the weaning period for about one-half the animals used (Table IV).

After 100 days on the test ration, animals were killed, and the heads were removed and fixed in 10 per cent neutral formalin. Following fixation, jaws were removed, stripped of soft tissue, and scored by the method previously described.[5]

RESULTS

Tables I, II, and III present in summary form the experimental regimens to which animals have been subjected, as well as the final data.

TABLE I

FEMALE	LITTER NO.	PRE-EXPERIMENTAL RATION	PREGNANCY RATION	WEANING RATION 0-21 DAYS	TEST RATION 21-121 DAYS	♀♀	INDIVIDUAL CARIES SCORES	♂♂	INDIVIDUAL CARIES SCORES	COMMENTS
39	M	PRC 74 d PLC 16 d	PLC	PLC	R2	0		4	0, 0, 0, 0	
34	L	PRC 71 d PLC 18 d	PLC	PLC	R2	0		4	0, 0, 0, 0	
6	O	PRC 63 d PLC 27 d	PLC	PLC	R2	0		3	0, 0, 0*	21-311 d on R2; 36 d on R4
8	P	PRC 61 d PLC 29 d	PLC	PLC	R2	0		4	0, 0, 0, 0	
20	Q	PRC 69 d PLC 30 d	PLC	PLC	R2	2	0,* 0*	3	0, 0, 0	34-154 d on R2; 21-154 d on R2
39	AB	PRC 23 d PLC 67 d	PLC	PLC	R2	2	0, 0	2	0, 0	
8	AF	PRC 14 d PLC 76 d	PLC	PLC	R2	2	0, 0	0		
6	AH	PRC 8 d PLC 82 d	PLC	PLC	R2	2	0, 0	3	0, 0, 0	
22	N	PRC 67 d R1 23 d	R1	R1	R2	0		3	0, 0, ¼	
23	T	PRC 55 d R1 35 d	R1	R2	R2	1	0*	2	0, 0	21-107 d on R2; 51 d on R100

41	U	PRC	54 d	R1	R2	R2	2	Slight cervical caries*	2	0, 0	21-109 d on R2; 227 d on R100, etc.
		R1	36 d								
46	V	PRC	50 d	R1	R2	R2	1	0	1	0	21-291 d on R2; 45 d on R4
		R1	40 d								
7	W	PRC	41 d	R2	R2	R2	2	0, 0	3	0, 0, 0	
		R1	40 d								
		R2	9 d								
40	X	PRC	37 d	R2	R2	R2	2	0, ½	2	0, ¼	
		R1	40 d								
		R2	13 d								
27	Z	PRC	32 d	R2	R2	H2	2	0, 0	2	0, 0	
		R1	40 d								
		R2	18 d								
23	AI	PRC	7 d	R2	R2	R2	2	0, 0	2	0, 0	
		R1	41 d								
		R2	42 d								
7	AP	R1	32 d	R2	R2	R2	1	0	0		
		R2	58 d								
20	AJ	PRC	6 d	R2	R2	R2	2	0, 0	0		
		PLC	67 d								
		R2	17 d								
41	AK	PRC	6 d	R2	R2	R2	1	0	2	0, 0*	21-299 d on R2; 18 d on R4
		PLC	40 d								
		R2	44 d								
48	AO	R2	90 d	R2	R2	R2	1	0	4	0, 0, 0, 0	

*See "Comments" column.

TABLE II

FEMALE	LITTER NO.	PRE-PREGNANCY RATION	PREGNANCY RATION	WEANING RATION 0-21 DAYS	TEST RATION 21-121 DAYS	♀♀	INDIVIDUAL CARIES SCORES	♂♂	INDIVIDUAL CARIES SCORES	COMMENTS
47	AW	PLC 90 d	R2	R2	R100	3	0, 0+*	1	0	Severe periodontosis with caries
60	AU	PLC 90 d	R2	R2 0-18 d R100 18-21 d	R100	0		1	¾*	21-354 d on R100
34	AT	PLC 74 d R2 16 d	R2	R2 0-13 d R100 13-21 d	R100	0		4	0, 0, 0*	Cervical caries; 13-527 d on R100
23	AX	R2 90 d	R2	R2 0-14 d R100 14-21 d	R100	4	0, 0, 0, 0	3	0, 0, 0	
48	AZ	R2 90 d	R2	R2 0-10 d R100 10-21 d	R100	1	0*	3	0, 0, ½*	21-168 d on R100
74	BA	R2 90 d	R2	R2 0-7 d R100 7-21 d	R100	3	½, ½, ¼*	1	Cervical caries*	124-277 d on PRC; 21-130 d on R100; severe periodontosis
79	AY	R2 73 d	R100	R100	R100	1	0*	3	0,*, 0,* ½	21-150 d on R100
60	BE	PLC 81 d R100 9 d	R100	R100	R100	1	0	4	0, ½, ½, ½	
139	BS	R4a 45 d R100 45 d	R100	R100	R100	4	0, 0, 0, 0	1	1	
48	BF	R2 ? d R100 ? d	R100	R100	R100	2	½, 0	2	0, ¼	
73	BB	R2 76 d R100 14 d	R100	R100	R100	2	0, ½	1	0	
113	BM	R2 56 d R100 34 d	R100	R100	R100	0		2	¼, 1	
120	BT	R2 43 d R100 47 d	R100	R100	R100	4	3, ½, ¼, 10*	4	1, 1, 2, 2	22-187 d on R100; 170 d on R4; 32 d on PLC
74	BU	R2 11 d R100 79 d	R100	R100	R100	4	0, ¼, 1, 1	2	½, 2	
120	CC	R100 90 d	R100	R100	R100	4	¼, 0, ½,* ¾*	4	1, ¼, 1,* ¾*	Maintained in Rochester, N. Y.
73	CI	R100 90 d	R100	R100	R100	0		3	67,* ½, 1	21-370 d on R100

*See "Comments" column.

In the tables, "Pre-experimental ration" refers to the rations consumed by the female for a ninety-day period prior to the beginning of the present study. If two rations are indicated, they are presented in the order of feeding followed by the number of days fed. In the presentation of caries scores, values of less than 1 have been reported as such. Other values have been rounded out to the nearest whole number. Under "Comments," additional information is presented that pertains to data recorded in the preceding horizontal columns.

Most animals reported have been on definite test regimens. However, the tables also include animals which have been used for breeding purposes and maintained on various experimental diets for long periods of time. Data from breeders are included to show that only moderate caries developed in some animals after extended periods and also that lesions were especially of the cemental and root surface type.

Table I presents data which indicate that animals born from mothers maintained on Purina Laboratory Chows or a purified ration supplying the mineral ash of one of these chows developed no caries in a 100-day test period. Under the experimental conditions described and with the group of animals employed, resistance to caries has been remarkably high, although the experimental ration contained 60 per cent sucrose. Animals retained this resistance for long periods as evidenced by the observation that those male and female animals used for breeding have been resistant to caries during an approximate one-year period. In other words, animals initially raised on chow and ash rations have shown no coronal enamel breakdown not only during a 100-day test period but also an additional period of exposure to subsequent high carbohydrate diets, for example, Rations 100, 4, and so forth, during which pregnancies were induced in females.

Table II includes data on animals which came under the influence of Ration 100 during various stages of pregnancy and weaning period, and which were subjected to a 100-day test period on this same diet. An examination of these data indicates that only lesions of minimal size were detectable. Although this finding is in contrast to the almost complete absence of caries demonstrated in Table I, the very small values, generally fractional, suggest that this diet, under the conditions of this study, is unsatisfactory as a means of inducing dental caries in hamsters.

Table III presents data from animals exposed to Ration 4 during the test period of 100 days. The prepregnancy ration and pregnancy ration included laboratory chows as well as Rations 1, 2, 100, 4, and 4a. After the experimental period of 100 days had ended, several of the animals were used for breeding purposes and continued on either Ration 4 diets or transferred to other rations as indicated. Animals were used for this purpose because the incidence of decay was very low and visual inspection of the maxillary teeth in live animals at 121 days of age showed little evidence of caries. All of the data in this group indicate that caries activity has remained very low even

TABLE III

FEMALE	LITTER NO.	PRE-PREGNANCY RATION	PREGNANCY RATION	WEANING RATION 0-21 DAYS	TEST RATION 21-121 DAYS	♀♀	INDIVIDUAL CARIES SCORES	♂♂	INDIVIDUAL CARIES SCORES	COMMENTS
39	AB	PRC 22 d PLC 68 d	PLC	PLC	R4a	0		2	1, 26	
34	AD	PRC 17 d PLC 73 d	PLC	PLC	R4a	1	¼			
38	AE	PRC 14 d PLC 76 d	PLC	PLC	R4a	0		1	120*	22-398 d on R4's
8	AF	PRC 11 d PLC 79 d	PLC	PLC	R4a	0		1	¼	
6	AH	PRC 5 d PLC 85 d	PLC	PLC	R4a	0		1	0	
47	AC	PLC 90 d	PLC	PLC	R4a	2	Cervical caries*	1	Cervical caries	21-188 d on R4a; 67 and 116 d on R100; 21-122 d on R4a; 183 d on R2; 28 d on R4
99	BN	PLC 90 d	PLC	R4a	R4a	1	½	1	½	
39	AQ	PLC 90 d	PLC	R4a	R4a	3	0, ¼, ¾	3	¾, ¼, ¼	
W-10	CU	PLC 90 d	PLC	R4	R4	1	0	2	¼, 0	
W-3	CV	PLC 90 d	PLC	R4	R4	0		4	0, ¼, 0, 6	
20	AJ	PLC 70 d R2 20 d	R2	R2	R4a	0		1	0	

23	A1	R1 R2	41 d 46 d	R2	R2	R4a	0		2	0, 0	
41	AK	R1 R2	40 d 45 d	R2	R2	R4a	2	0,+*	1	5	21-99 d on R4a; 218 d on R100; 21-246 d on R4a; cervical caries
7	AP	R1 R2	31 d 59 d	R2	R2	R4a	0		3	1, ¼, ¼	
99	CF	PLC R100	47 d 43 d	R100	R100	R4	2	1, ¼	1	0	
100	CG	R100	90 d	R100	R100	R4	2	¼,* ¼	2	1,* 69*	21-316, 21-238, 2-387 d on R4
39	BC	PLC R4a	74 d 16 d	R4a	R4a	R4a	2	½, ½	2	2, ¼	
41	AV	R2 R4a	73 d 17 d	R4a	R4a	R4a	3	½, ½, ½	4	2, 2, 6, 28	
156	BW	R100 R4	61 d 29 d	R4	R4	R4	2	Slight cervical caries*	0		21-126 on R4; 184 d and 253 d on chows
155	BY	R100 R4	24 d 66 d	R4	R4	R4	2	3, 4	1	25	
107	BR	R4a	90 d	R4a	R4a	R4a	0		5	0, ½, ¼,* ½,* ¼	21-50 d on R4a; 78 d on R4; 21-50 d on R4a; 200 d on R4
156	CL	R4	90 d	R4	R4	R4	2	4,* 1*	0		21-133 d on R4
155	CN	R4	90 d	R4	R4	R4	2	2, 10	2	2, 66*	21-128 d on R4; 54 d on PLC
315	CT	R4	90 d	R4	R4	R4	1	52*	1	75*	21-147 d on R4; 143 d on chows; 21-290 d on R4

*See "Comments" column.

in animals which lived well beyond the 100-day experimental period. It is of interest to note, however, that the carious process is somewhat more active in these animals than in those on other rations reported.

Table IV presents information relative to reproduction and growth on the rations employed. At the beginning, some difficulty in inducing pregnancy seemed to be experienced with the purified or semipurified diets. When animals are transferred from a stock diet such as one of the laboratory chows to a ration of R100 type, they may lose weight and not reproduce readily. However, as with other rations, there is variability in this regard, and it appears that once animals have become accustomed to a particular ration, reproduction is not too difficult to obtain if the diet is not grossly inadequate and precautions are taken.

TABLE IV

DIET	LITTERS BORN	LITTERS DESTROYED	LITTERS WEANED	NO. OF PUPS	AVERAGE NO. OF PUPS	AVERAGE WEANED WEIGHT AT 21 DAYS	AVERAGE WEIGHT AT APPROX. 50 DAYS	AVERAGE WEIGHT AT APPROX. 121 DAYS
Laboratory chow	32	4	11	52	4.7	36.3 ± 3.4	87.1 ± 6.4	
With carrots			17	97	5.7	38.5 ± 6.6	84.1 ± 8.5	
Ration 100	20	6	7	26	3.8	28.3 ± 4.6	72.9 ± 11.4	98.8 ± 18.4
With carrots			7	40	5.7	28.3 ± 4.2	72.2 ± 6.2	89.2 ± 7.1
Ration No. 2	20	5	15	75	5.0	32.2 ± 4.1	79.6 ± 8.9	100.1 ± 13.7
Rations 4 and 4a	30	10	8	26	3.3	29.2 ± 8.3	77.1 ± 17.5	104.4 ± 18.7
No. 4 with carrots			12	43	3.6	29.8 ± 8.4	68.1 ± 17.6	101.8 ± 17.0

When breeders are on a powdered diet of the R100, R2, or R4 type, it is necessary to pay special attention to litters from the seventh to eighth days until weaning time. Since it is necessary to provide food for the young pups during this period, some of the ration must be dumped on the floor of the cage where the pups can get to it or supplied in the form of small pellets. The pellets can be prepared by mixing the ration with a small amount of water and then pressing the mixture into a thin waferlike mass which will dry in air. Care must be taken with the high-sugar diet lest too much water be used with the result that the pellets become too hard. All diets are stored in an icebox. I have successfully raised animals on all the rations without the use of supplements such as fresh carrots. To do this requires rather careful personal supervision and constant attention to many details. The introduction of fresh carrots during the weaning period from 7 to 21 days minimizes the detailed care needed for animals raised without such a supplement and is an almost necessary expediency when one needs to rely on auxiliary personnel for the care and feeding of animals. The data suggest that the number of animals successfully weaned may be greater when the litters are supplemented with the carrots.

The best reproduction and growth have been obtained when animals have been raised on laboratory chow. Ration 2 is superior to Rations 100 and 4 as

evidenced by the 21-day and 50-day weights. Reproduction on Ration 4 or 4a has not been especially good. There seems to be little difference on the basis of data or actual experience between Ration 100 and Ration 4 in so far as reproduction is concerned.

An observation of interest is the apparent lack of more rapid growth for female animals. In most laboratories, female hamsters have grown more rapidly than male animals, and in previous studies this has been a very consistent finding. For the past three years, however, there has been no difference in growth between the sexes apparent at 21 days, 50 days, or 121 days (Table V). Healthy male and female animals which achieve a 140-gram weight have occasionally developed on these rations.

TABLE V

RATION	21-DAY WEIGHTS		50-DAY WEIGHTS		121-DAY WEIGHTS	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Purina Laboratory Chow	36.4 ± 4.8	35.6 ± 5.1	91.5 ± 8.3	85.4 ± 7.8		
Ration No. 100	27.2 ± 4.4	28.7 ± 3.2	73.7 ± 8.1	71.4 ± 7.7	95.2 ± 11.0	94.6 ± 15.3
Ration No. 2	32.0 ± 3.7	31.4 ± 4.5	78.6 ± 8.0	75.4 ± 8.8	97.2 ± 8.7	92.5 ± 13.4
Ration No. 4	29.0 ± 9.0	32.0 ± 8.4	77.0 ± 16.3	74.7 ± 8.2	99.7 ± 25.4	105.8 ± 11.5

The data between male and female animals in Table V have not been tested for significance because the average weights are so obviously close and the standard deviations overlap to such a wide degree.

When data on matings are compared to the actual number of pregnancies which go to full term, it appears that about one-half the matings do not result in successful pregnancies. For example, of approximately 336 presumably timed matings, only 171 litters have been born. It is not uncommon to have a female develop all the signs of pregnancy and fail to deliver. Animals have not been sacrificed to determine the exact cause of this phenomenon, but it suggests the development of pregnancy followed by resorption of the fetuses in utero. This reaction is less frequently observed in animals on chow diets. As has been reported elsewhere by others, some females destroy their litters or reproduce poorly regardless of the ration employed. Other animals reproduce well even under obviously difficult circumstances.

DISCUSSION

The unexpected degree of caries resistance, as evidenced by the small extent of molar tooth destruction after 100 days on an experimental diet, has been disappointing as well as inexplicable. These findings are in marked contrast to those reported a few years ago in which rampant decay was observed in hamsters raised on Purina Rabbit Chow Checkers and transferred at about 30 to 40 days of age to diets similar to Ration 4.

In retrospect, Ration 2 might not have been expected to induce caries if the same results apply to hamsters as have been found in white rats. Subsequent to the completion of hamster tests with this ration, Sognnaes and Shaw[8] have reported reduced caries susceptibility in rats fed diets containing Purina

Laboratory Chow ash. Before these results were known, this diet was tried in hamsters because of what appeared to be poor breeding performance on Ration 1. After the diet was changed from Ration 1 to Ration 2, breeding seemed to improve. This response was probably due in part to adaptation of breeders to the purified rations. It does appear, however, that the ash ration is superior to the purified salt one, as comparison of data on reproduction and weaned weights of animals on Ration 100 (which is very comparable to Ration 1) and Ration 2 indicates better litter size and superior 21-day weights on the ash ration. The average 21-day weight on Ration 100 was 28.3 ± 4.4 grams, on Ration 2 32.2 ± 4.1 grams (Tables IV and V).

A matter of considerable surprise was the very low cariogenic activity in animals fed Ration 100 for long periods of time. Since this diet has proved to be conducive to caries susceptibility and caries activity in cotton,[6] Long-Evans,[9] and albino rats,[10] it was hoped that if hamsters could be adapted to breed and grow successfully on Ration 100, or a somewhat comparable purified diet, cariogenic activity would result. The caries-inhibitory mechanisms, either internal, external, or both, in operation are not known.

When it became apparent that neither Ration 2 nor Ration 100 was conducive to caries activity, an attempt was made to induce decay by using diets composed of natural foodstuffs and similar to those previously employed, that is, Rations 4 and 4a.

The results of this dietary modification were equally surprising in that although there was a slight increase in caries activity, the extent of decay was very much less than that previously observed. Only in a few animals maintained on Ration 4 for long periods of time was decay seen, generally of the cementum and root surface.

As previously mentioned, this low cariogenic activity is difficult to explain. Among possible factors which either singly or in combination might account for these results, one thinks of (1) the presence of some unrecognized inhibitory agent or agents either in the diets or otherwise, (2) the adaptation of the animals to the environment so that their internal and external environment has been changed, (3) mechanisms influenced by genetic factors, (4) possible changes in bacterial flora, and so forth.

The fluoride content of the rations is not believed to be high except possibly for the Purina Laboratory Chow with reported fluoride content of 21 ppm and the Purina Rabbit Chow Checkers with from 15 to 80 ppm. Ration 2 has a calculated fluoride content of approximately 7 ppm; Ration 100, approximately 1.5 ppm; and Ration 4, approximately 2 ppm.

The possibility exists that an environmental factor or factors might be causing reactions in the animals which influence caries activity. To test, in preliminary fashion, this possibility, litter No. 120 CC on Ration 100 was divided. Two males and two females were maintained in the controlled environment of the animal house of the Harvard School of Dental Medicine, Boston, while two males and two females were sent to Rochester, New York, and maintained by Dr. J. W. Hein in the nonregulated environment of his laboratory. The ration was prepared in Boston and sent to Rochester. There was no apparent difference in the results of this small test.

Observations of genetic factors which may influence susceptibility and resistance in dental caries in white rats have been made by Hunt, Hoppert, and Erwin.[11] Recently somewhat comparable findings have been made by Hodge, Johansen, Hein, and Maynard[12] in hamsters. That all animals tested in this study were descendents of one female suggests that a resistant line may have been established. However, if the mechanisms which account for the high caries resistance in these hamsters were due primarily to factors controlled genetically, one might reasonably anticipate more variability than has been observed. The fact that caries activity has been essentially negligible with all rations tested suggests highly dormant inherited mechanisms or a combination of factors.

SUMMARY

Dental caries has remained essentially nonactive in a group of over 221 Syrian hamsters tested with several high carbohydrate regimens. Reasons for the unexpectedly low caries activity are unknown.

I wish to acknowledge the assistance of Dr. John W. Hein, who was kind enough to maintain one-half of litter 120 CC in his laboratory for 100 days.

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EXPERIMENTAL DENTAL CARIES

III. The Effect of Estradiol and Diethylstilbestrol on Dental Caries, Fluorine Metabolism, and the Salivary Glands of Intact and Gonadectomized Rats

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THE studies to be reported here are a portion of the series which has been designed in an attempt to determine how the endocrine organs and their various products affect experimental dental caries and to clarify the relationship of these organs to the salivary glands and fluoride metabolism. Considerable evidence exists[1-3] to warrant further investigation of this problem. Junqueira, Rubinovitch, and Fajer[4] have shown enzymatic differences in the salivary glands of male and female mice. Sreebny[5] has shown enzymatic alterations as well as histologic changes in the submaxillary gland of the rat following hypophysectomy. Rathje[6] has suggested that the action of fluorine in reducing dental caries may be by reduced sympathicotony of the salivary glands mediated through a competition of the thyroid gland for fluorine and iodine essential to its production of hormone, such that a larger quantity of more liquid saliva is produced, thereby increasing the resistance to dental caries.

The studies reported from this laboratory have not been designed to measure quantitatively such differences produced by gonadectomy, desalivation, and the administration of sex hormones, but rather to determine initially whether alterations of various types occur. Future investigations may be channeled along more exacting lines after these facts have been established, with the aid of conventional or newer and more critical technics.

EXPERIMENTAL

The studies to be reported, although similar in nature, will be treated separately since they were not carried out simultaneously. In the first study (Experiment I), a total of 144 albino rats,* of which 131 completed the experimental period, were divided as equally as possible according to sex, littermates, and initial weight into eight groups. Two groups of each sex consisted of control animals and a series which had been gonadectomized. A third group of each sex were unoperated animals which received an injection of 1.5 mg. of diethylstilbestrol† per week, subcutaneously. The fourth group of each sex had a 0.25 Gm. pellet of estradiol† implanted subcutaneously at the beginning

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*Sprague-Dawley strain, supplied by Carl Wilson, Beech Grove, Indiana.

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of the study. All animals were started on the experiment at 38 days of age, and the investigation had a duration of 119 days. The caries-producing diet[7] and distilled water were supplied ad libitum.

At sacrifice, the heads were prepared for examination, as reported previously.[8] In addition, the pellets of estradiol remaining were removed and weighed. It was found that the average weight of the remaining estradiol pellets in the male was 0.197 Gm. and in the female, 0.209 Gm., thus indicating that, during the experimental period, the males absorbed an average of 0.053 Gm. of estradiol while the females absorbed an average of 0.041 Gm. of estradiol per animal. The uteri and testes were removed, weighed, and fixed for microscopic examination along with the following structures: salivary glands, tongue, thyroid, parathyroid, pancreas, adrenal, and a long bone. A femur from each of six representative animals in each group was also taken for calcium, phosphorus, and fluorine analysis.

The second study (Experiment II) consisted of a total of 144 albino rats, of which 127 completed the experimental period, and were divided into eight groups similar to those described previously, except that the animals receiving the diethylstilbestrol and estradiol were gonadectomized. The control and gonadectomized groups were treated identically to those in Experiment I. In this second study, one change was made in the method of administration of the estradiol. Instead of the pellets being implanted, the estradiol was dissolved in cottonseed oil and 1.5 mg. was injected subcutaneously once each week. The animals began the experiment at 34 days of age, and the experimental period lasted for 130 days. As before, the caries-producing diet and distilled water were available ad libitum. At sacrifice, the heads were prepared in the same manner and the same tissues were removed for histological examination and chemical analysis.

DATA AND DISCUSSION

The data pertaining to the caries incidence in the animals receiving the natural and the synthetic female hormone (Experiment I) are seen in Table I. In the males, castration appears to decrease the incidence of dental caries, while in the females, caries appears to be increased, which is in keeping with previous work[1] reported from these laboratories. In the males, the administration of diethylstilbestrol decreases caries, and estradiol increases the caries experience, while in the females, both the artificial and the natural female hormone increase caries experience. Previous work[1] indicated that diethylstilbestrol decreases caries experience in unoperated females, so the findings reported herewith do not corroborate these findings. However, there were some differences in the method of administration of the synthetic female hormone in the two experiments, but it is not understood at the present time how this might modify the findings. In the previous studies,[1] the diethylstilbestrol was administered in a sesame oil vehicle, and encapsulation at the injection site was frequently observed. In order to alleviate this occurrence, cottonseed oil was substituted and much less encapsulation was noticed. Theoretically, in

the former studies much less hormone may have been absorbed, while in the present studies the level of diethylstilbestrol administered and absorbed undoubtedly was extremely high.

TABLE I. A COMPARISON BETWEEN THE CARIES EXPERIENCE IN UNOPERATED RATS RECEIVING DIETHYLSTILBESTROL AND ESTRADIOL

GROUP	NUMBER OF ANIMALS	FINAL WEIGHT (GM.)	NUMBER OF CARIOUS LESIONS	EXTENT OF LESION	S.I.P.*	CARIES CHANGE† (%)
			Males			
Controls	16	242	6.3	2.24	14.1	—
Castrates	18	215	5.1	2.10	10.7	-19
Control + DES	16	154	5.2	2.26	11.8	-17
Control + estradiol	16	156	9.2	2.59	23.7	+46
			Females			
Control	18	191	5.8	2.42	14.0	—
Castrate	17	217	7.0	2.38	16.6	+21
Control + DES	17	148	8.1	2.60	21.1	+40
Control + estradiol	15	148	8.0	2.45	19.6	+38

*S.I.P.—severity index product.
†Using number of lesions to compute difference.

When the size of the lesions and their number are evaluated by one factor, S.I.P. (severity index product), instead of using only the caries experience as evaluated by the number of lesions, the same relative caries effect is noted. However, the size of the lesion does not compare exactly with the number of lesions. For example, in the control males there is an extent value of 2.24 which corresponds to a mean number of 6.3 lesions per animal, but in the control males receiving diethylstilbestrol there is a reduction in the number of lesions by approximately 20 per cent, while the size of the lesions appears to be about the same. Again, in the female controls there is an extent value of 2.42 which corresponds to a mean number of 5.8 lesions per animal, while in the ovariectomized animal there is an increase in caries experience by approximately 20 per cent, but the size of the lesions is essentially the same. Thus, it does not seem plausible in every instance to intimate that the size of the lesion is directly proportional to the number of initial lesions. A statistical evaluation of the data in Experiment I indicates that the only significant difference in the males is between the control and the controls receiving estradiol. In the females, both the controls receiving diethylstilbestrol and those receiving estradiol are significantly different from the female controls. The probabilities that the differences could occur by chance in the former groups are 35 and 49 out of 10,000 in the diethylstilbestrol and estradiol groups, respectively.

Table II indicates the caries experience in the gonadectomized animals receiving the synthetic and the natural female hormones (Experiment II). It has previously been found[1] that orchiectomy decreases caries experience and ovariectomy increases it, but in this experiment ovariectomy decreased the dental caries incidence in the rat. The decrease in caries in the female is not in keeping with previous work,[1] and at the present time it is not possible to explain this finding. When the males were orchiectomized and diethylstilbestrol or estradiol was administered, there was an increase in caries, and this

may reasonably be justified since the female controls receiving the identical hormones have a marked increase in caries experience. In the ovariectomized animals receiving hormones, an increase in caries is found, the effect being somewhat more pronounced in the animals receiving diethylstilbestrol than in those receiving estradiol. These findings corroborate previous data.[1] Again, in this series, the S.I.P. parallels initial caries experience as evaluated by examining the mean number of lesions per animal, but the extent of the lesion is not dependent quantitatively upon initial caries attack. A statistical evaluation of the caries experience in Experiment II indicates a significant reduction in caries in the castrate males as compared to the controls. The probability was only 17 times in 10,000 that this reduction could occur by chance alone. In the females the difference between the controls and the castrates receiving diethylstilbestrol is also significant, with a probability of 44 times out of 10,000 that this number could occur by chance alone. None of the other findings are statistically significant; however, this does not discredit the findings, since such differences as were found in these studies might be of real importance from a biologic viewpoint were the experiments conducted for longer periods of time.

TABLE II. A COMPARISON BETWEEN THE CARIES EXPERIENCE IN GONADECTOMIZED RATS RECEIVING DIETHYLSTILBESTROL AND ESTRADIOL

GROUP	NUMBER OF ANIMALS	FINAL WEIGHT (GM.)	NUMBER OF CARIOUS LESIONS	EXTENT OF LESION	S.I.P.*	CARIES CHANGE† (%)
			Males			
Control	16	288	8.6	2.21	19.0	—
Castrate	16	259	6.7	2.24	15.0	-22
Castrate + DES	17	158	9.6	2.47	23.7	+12
Castrate + estradiol	16	180	9.5	2.48	23.6	+10
			Females			
Control	16	183	8.9	2.35	20.9	—
Castrate	17	196	7.5	2.25	16.9	-16
Castrate + DES	16	144	10.6	2.24	23.8	+19
Castrate + estradiol	16	159	9.7	2.53	24.5	+ 9

*S.I.P.—severity index product.
†Using number of lesions to compute difference.

Table III indicates the skeletal analysis for fluorine, calcium, and phosphorus. There appears to be no noticeable differences in fluorine storage except possibly in those animals receiving diethylstilbestrol or estradiol. In these instances there is a somewhat greater concentration in both the males and females when compared to the respective control animals, but the total amount is slightly less in the males. In the females the diethylstilbestrol is less and the estradiol is more. This is an unexpected finding for two reasons: First, the animals receiving the synthetic and natural hormone are considerably smaller in size and, thus, one would expect that they would ingest less food and then obviously store less of the element. However, this is apparently not true in all instances, for in the case of the female control receiving estradiol, the animal is considerably smaller, as judged by final weight and the size of the femur, yet there is a noticeable increase in both the concentration and the total amount of fluorine in the femur. Second, the animals with

the greatest amount of fluorine could be expected to have the greatest caries resistance, but if the female controls receiving estradiol are compared with the control animals it is apparent that the animals having the greater amount of fluorine in the skeleton also have greater evidence of caries activity. From this and other evidence,[9] it is difficult to postulate a rule that would indicate a positive correlation between fluorine storage in the skeleton and the incidence of dental caries in the rat. An interesting finding which has been noticed repeatedly before[8-10] is that female controls have a higher concentration and frequently a greater total amount of fluorine in the skeleton than do comparable males. This is difficult to explain since the female is a smaller animal and ingests considerably less food than the male.

TABLE III. ANALYSIS OF REPRESENTATIVE FEMURS IN UNOPERATED ANIMALS RECEIVING DIETHYLSTILBESTROL AND ESTRADIOL

GROUP	DESSICATED WT. (GM.)	ASHED WT. (GM.)	ASH (%)	F CONCENTRATION (μG/GM.)	TOTAL F (MG.)	CALCIUM (MG. %)	PHOSPHORUS (MG. %)
			Males				
Control	0.5761	0.2958	50.7	162.2	0.0482	38.6	19.3
Castrate	0.5145	0.2366	45.9	160.3	0.0379	38.2	19.2
Control + DES	0.4657	0.2284	48.9	194.4	0.0442	38.6	19.3
Control + estradiol	0.4487	0.2305	51.3	201.3	0.0460	38.7	19.9
			Females				
Control	0.5232	0.2620	50.0	199.9	0.0521	38.3	19.9
Castrate	0.4682	0.1764	48.8	191.0	0.0431	38.9	19.2
Control + DES	0.3585	0.1721	48.3	230.8	0.0397	38.6	19.0
Control + estradiol	0.4486	0.2480	52.8	261.9	0.0640	36.9	19.0

TABLE IV

ANALYSIS OF REPRESENTATIVE FEMURS IN GONADECTOMIZED ANIMALS RECEIVING DIETHYLSTILBESTROL AND ESTRADIOL

GROUP	DESSICATED WT. (GM.)	ASHED WT. (GM.)	ASH (%)	F CONCENTRATION (μG/GM.)	TOTAL F (MG.)	CALCIUM (MG. %)	PHOSPHORUS (MG. %)
			Males				
Control	0.7968	0.3459	43.2	102.7	0.0353	38.0	18.9
Castrate	0.7619	0.2998	39.2	116.7	0.0347	37.4	19.8
Castrate + DES	0.5144	0.2383	46.5	155.6	0.0372	37.2	19.8
Castrate + estradiol	0.5868	0.2768	47.5	132.1	0.0371	37.1	19.4
			Females				
Control	0.6448	0.2661	41.5	150.4	0.0405	—	—
Castrate	0.5828	0.2295	39.6	118.8	0.0272	37.8	19.6
Castrate + DES	0.5053	0.2389	47.3	94.8	0.0227	37.2	20.0
Castrate + estradiol	0.6522	0.2550	39.3	170.8	0.0431	37.6	19.6

Table IV demonstrates the fluorine storage in the femur of the gonadectomized rats receiving either diethylstilbestrol or estradiol (Experiment II). In the males, some differences in fluorine storage are noted in the two groups which were orchiectomized and which received diethylstilbestrol or estradiol. In these two groups there appeared to be a tendency to store somewhat larger amounts of the element. This result is reminiscent of that in the control males receiving these same two substances. However, in the females, the ovariectomized animal receiving diethylstilbestrol stored considerably less fluorine

than the controls, while similar animals receiving estradiol stored somewhat more. Moreover, ovariectomized animals appear to store less fluorine than control females, while a similar tendency seems to hold true with orchiectomized animals, but to a smaller degree. The status of fluorine retention in these and similar experiments seems to be so intimately associated with growth processes that it is difficult to explain these findings clearly until more information is available concerning the absorption and metabolism of fluorine under such varied conditions as are operating in these studies.

Histologic Studies.—Histologic examination of the salivary glands has re-emphasized the fact that both gonadectomy and the administration of either estradiol or diethylstilbestrol cause marked alteration in their structure, particularly evident in the submaxillary gland.

In both experiments reported, it has again been shown that orchiectomy and ovariectomy produce a marked diminution in both the size and number of granular tubules of the submaxillary gland (Table V). Although the direct measurement of these tubules indicates this alteration in structure, the true differences which exist cannot be appreciated without actually visualizing the histologic sections. The reason for this lies in the fact that in the control animals, these secretory tubules contain numerous large granules in the acinar cells, whereas in the gonadectomized animals, there is an extremely marked loss of these granules but seldom a complete loss. Therefore, even though the cells making up the acini contain just a few granules, their diameter is measured and their number per area is determined; still, the presence or absence of these granules cannot be measured and recorded with any degree of accuracy.

TABLE V

THE EFFECT OF ORCHIECTOMY AND OVARIECTOMY AND THE ADMINISTRATION OF ESTRADIOL AND DIETHYLSTILBESTROL ON THE SIZE AND NUMBER OF GRANULE-BEARING TUBULES IN THE SUBMAXILLARY GLAND OF THE RAT

	AVERAGE NO. OF TUBULES PER UNIT AREA*	STANDARD DEVIATION	AVERAGE DIAMETER OF TUBULES (MICRONS)	STANDARD DEVIATION
Control males	16.5	4.6	42	2.2
Gonadectomized males	8.1	6.3	36	2.2
Control males + DES	11.1	6.9	37	2.2
Control males + estradiol	6.5	2.1	36	2.2
Control females	12.2	4.8	40	2.8
Gonadectomized females	11.8	2.6	37	2.2
Control females + DES	7.2	4.9	35	1.4
Control females + estradiol	10.5	4.7	37	2.0

*Area approximately 0.255 sq. mm.

A second interesting finding has been that both diethylstilbestrol and estradiol, whether administered to intact or gonadectomized male or female animals, cause alterations in the submaxillary glands which are indistinguishable histologically from those changes induced by gonadectomy alone.

In the present studies, it has not been possible to correlate the histologic alterations in the salivary glands with the caries experience as induced by

gonadectomy or the administration of the sex hormones. Although the latter two procedures produce a response in the salivary glands which appear histologically identical, the effect on the caries experience of these procedures appears, in general, to be diametrically opposed to each other. At the present time, the explanation for this finding is not evident.

SUMMARY

1. In two separate studies, it has been shown that the incidence of experimental dental caries in orchiectomized rats was considerably lower than in control animals. Ovariectomized rats in one study also exhibited reduced caries incidence, while in a second study an increase in caries was noted.

2. The administration of both diethylstilbestrol and estradiol to control and gonadectomized animals resulted in an increased incidence of dental caries in all cases except in the group of intact males receiving diethylstilbestrol.

3. The fluoride concentration in femurs of intact males and females receiving the two hormones was appreciably higher than that of corresponding control animals. The same finding held true for gonadectomized males and females except in the case of ovariectomized females receiving diethylstilbestrol. Gonadectomy per se did not appear to influence the fluorine concentration.

4. The histologic structure of the submaxillary gland was markedly altered by gonadectomy and administration of the two female sex hormones. This finding could not be correlated with the alterations in caries incidence.

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A MICRO-ANTIMONY ELECTRODE DESIGNED FOR INTRAORAL pH MEASUREMENTS IN MAN AND SMALL EXPERIMENTAL ANIMALS

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ALTHOUGH the glass electrode is more reliable than the antimony electrode for measuring pH, the antimony electrode has been considered more suitable for pH measurements in the mouth because it has been found to be sturdier and can be made smaller than the glass electrode.[1] It has not been possible to construct antimony electrodes sufficiently small to gain access to small carious lesions and interproximal spaces. The difficulty in making minute electrodes has been that the cast antimony sticks which have been used tend to fracture because of brittleness. This limitation has been overcome by enclosing the antimony metal in a glass capillary.[2, 3] Recently a method has also been reported for making a micro-antimony electrode involving deposition of antimony on a platinum wire.[4] It is the purpose of the present paper to describe the construction of a micro-antimony electrode and also to present some intraoral results obtained with it in man and the hamster.

CONSTRUCTION

In making the antimony electrode, advantage is taken of the fact that Pyrex glass becomes soft at the melting point of antimony. Therefore, when a piece of glass tubing containing antimony is heated until the metal liquefies, the tube containing the molten metal may be drawn to any desired dimension. Because the antimony expands during solidification, the lumen of the glass tubing is well packed with the antimony. The glass tubing serves to protect the enclosed minute amount of antimony and adds appreciable strength. Only a pure grade of antimony metal can be used in th construction of a micro-antimony electrode. Unpurified antimony has invariably given disappointing results. In the present work, the antimony was purified by electrolysis according to the method of Schuman,[5] and kept in a closed glass container until used.

Electrodes for intraoral pH measurements are made conveniently in any shape from a Pyrex tubing of about 7 mm. outer and 2 mm. inner diameter. A schematic drawing of an electrode shaped like a dental probe is shown in Fig. 1. The tubing is first drawn to a desired form and length, care being taken to maintain an inner diameter sufficiently large to allow for insertion of a silver wire about 0.35 mm. thick. This wire will be inserted later and will serve as a lead to the pH meter. A small amount of powdered antimony (2 to 3 mm.) is now introduced into the glass tubing and tapped toward the drawn end. The tubing is heated over a flame until the antimony is melted, and the metal is induced to slide gently to the end of the capillary lumen of the tubing.

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The capillary containing the antimony is reheated and moulded in the desired shape. A small Bunsen burner adjusted to give a sharp flame is well suited for this work. The flame should not be too hot because overheating of the antimony causes bubble formation when the metal solidifies.

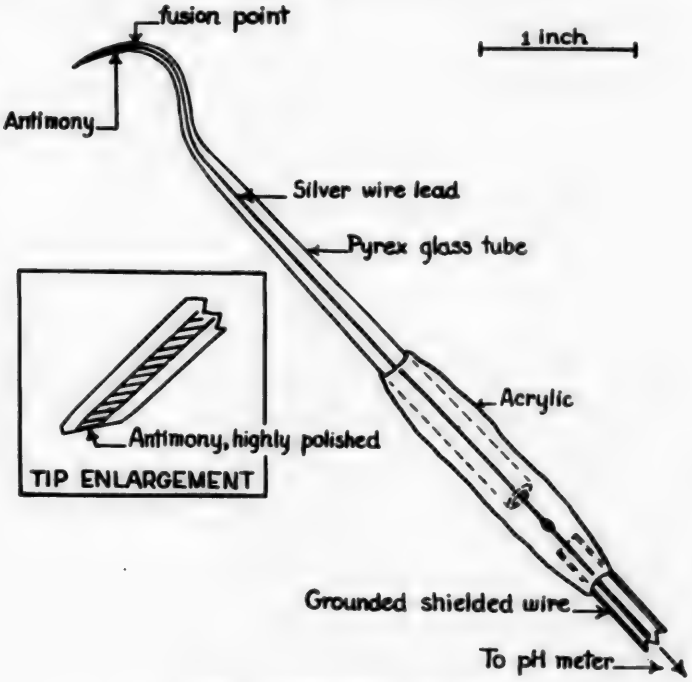

Fig. 1.—Probe-shaped electrode.

The 0.35 mm. silver wire, which has previously been soldered to a shielded cable (copper wire, multistrand, tinned, obtained from Gavitt Manufacturing Company, Inc., Brookfield, Massachusetts), is introduced into the tubing and, by gentle heating, it is fused with the antimony. Since the silver wire melts into the antimony if heated too long, it is again important to avoid application of too much heat. A small amount of steel soldering paste applied to the silver wire has been found to improve the bonding. As the antimony silver junction is easily broken because of the fragility of the antimony metal, it is necessary to immobilize the silver wire at the open end of the glass tubing. This is conveniently achieved by sealing the glass tubing with self-curing acrylic to prevent movement of the wire within the glass tubing.

The capillary end of the glass tubing is now worn down with fine cuttlefish disks until the antimony is exposed. It has been found that steadier pH readings have been obtained when the end of the electrode is finished with a slight slant, as shown in the insert in Fig. 1. This has a twofold effect: (1) During a pH reading, the glass of the electrode end will contact the hard

structure of the tooth surface, thus permitting the antimony to contact only the plaque material or the liquid phase on the surface. (2) The slanting will increase the surface area of exposed antimony metal.

Although there appears to be no space between the antimony and the glass, it is advisable to dip the tip of the electrode in melted wax in order to prevent possible seepage of electrolyte between the antimony and the glass. The electrode is finally polished with crocus disks and is ready for use. A high polish of the antimony metal is absolutely essential, as electrodes with a scratched or rough metal surface have consistently given faulty readings. Between tests the electrode should be kept in air since unreliable readings are obtained after prolonged exposure to water. It is advisable to repolish the electrode before tests.

An electrode made from Pyrex glass, as specified, has considerable strength because of the thickness of the glass walls at the working end of the electrode. Successful measurements have been obtained in the mouth with an electrode of this type having an outer diameter at the working end of 0.6 to 0.7 mm. Although more finely pointed electrodes may be obtained by using thinner-walled glass tubing, these electrodes do not have sufficient rigidity to be used in the mouth.

INTRAORAL MEASUREMENTS

As a reference electrode, either a Beckman calomel electrode No. 14340, which may be placed directly in the mouth, or a conventional calomel electrode connected to the mouth through a saturated KCl bridge has been used. The pH readings have been taken on a Beckman pH meter, industrial model M, with a battery and a variable resistor interposed between the calomel electrode and the meter.[6] Electrodes which have been properly constructed will give pH readings with a deviation of 0.2 pH unit or less on standard buffers in the range pH 4 to pH 7. It is necessary that the operator of the instrument, the person who manipulates the microelectrode, and the patient be grounded during a test. For satisfactory readings it is also important that movement of the antimony electrode on the tooth surface be avoided. In most instances, good readings were obtained within a few seconds when these precautions were taken. During a test it is advisable to standardize the electrode against a known buffer at intervals of ten to fifteen minutes, because there may be slight changes in the system from time to time.

APPLICATIONS OF THE ELECTRODE

A number of intraoral pH measurements have been taken with the micro-antimony electrode in areas inaccessible to the glass and other more bulky electrodes. These areas include interdental spaces, different locations on the same tooth, carious lesions, and gingival pockets.

Some interesting observations have been made: The debris in interdental spaces was frequently found to be alkaline, ranging from pH 7.0 to 7.8; even higher pH readings, up to 8.6, were found in debris on labial tooth surfaces. It was observed repeatedly that the pH may differ considerably in two adjacent

spots on a tooth surface. In one instance, an upper incisor with considerable debris had a pH reading of 8.5 close to the gingival margin and 7.1 a small distance from the gingival margin.

Low pH readings were frequently observed in carious lesions, which is in agreement with Stephan's findings.[1] Our readings were in the range of 6.8 to 4.7. A pH of 4.7 was found in a cavity which extended into the dentin but which had a very narrow opening. After application of 5 per cent glucose solution, the pH decreased to 4.1.

A few pH measurements taken in gingival pockets gave readings from 7.4 to 7.8. However, readings obtained from gingiva were frequently unstable because of the difficulty of keeping the electrode motionless during the test. It is known that motion of the antimony electrode in relation to the electrolyte will also influence the potential of the electrode and introduce an error in the pH reading.[7]

The problem of motion likewise made salivary measurements in the mouth difficult. The pH readings, which were considered stable, were found to vary from 6.5 to 7.2. It is interesting to note that when the point of the electrode was brought in contact with the mucous membrane, again involving motion of the electrode, higher readings (up to 8.9) were often obtained.

The micro-antimony electrode may be used for measuring pH on the teeth in small experimental animals. A few intraoral pH readings were obtained in the Syrian hamster. The jaws were immobilized according to the method of Johansen,[8] and the reference electrode was placed in the animal's pouch. The pH in the hamster's mouth is generally higher than in the human mouth. The pH of tooth surfaces ranged from 7.4 to 8.5. In a carious lesion which was dried before the test, the pH was 6.7. After application of sugar solution, it fell to 5.6. Measurements of this type in different kinds of caries-susceptible animals may establish whether there is a common mechanism in development of dental caries.

Most of the micro-antimony electrodes we have used have given stable readings, but very minute ones are unreliable. Therefore, it is generally advisable to construct electrodes as large as accessibility will permit. Occasionally electrodes were inactivated by debris on the tooth surface and had to be repolished before further use.

It is concluded that the micro-antimony electrode permits pH readings of areas which are inaccessible to other known types of electrodes. Many of the readings obtained with this instrument are within the range reported by workers using other types of electrodes. However, our higher pH readings obtained in debris should be confirmed before being accepted.

SUMMARY

1. A method is described for making a micro-antimony electrode suitable for measuring pH in interproximal areas and carious lesions in teeth of man and experimental animals.

2. Preliminary intraoral pH measurements on human and hamster teeth are given.

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EVALUATION OF THE MICRO-ANTIMONY ELECTRODE

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THE determination of pH in carious lesions obviously necessitates the use of a small sturdy electrode. It is possible to construct micro-antimony electrodes[1-3] which meet this requirement, but results obtained with these instruments have not been critically evaluated. The purpose of the present paper is to compare intraoral and in vitro pH readings obtained with a glass and a micro-antimony electrode and to evaluate some of the factors which may influence the behavior of the antimony electrode in the mouth.

There is considerable evidence that a large antimony electrode will give satisfactory intraoral pH measurements. For instance, the pH readings obtained by Stephan[4] with the antimony electrode on plaque material in situ agree well with those obtained by Fosdick with the glass electrode on plaque material in vitro.[5] Stephan[4] and Stralfors,[6] on the basis of extensive clinical trials, have expressed the opinion that the antimony electrode gave pH readings on tooth surfaces with an accuracy within 0.2 pH unit. Fosdick also stated that the antimony electrode gave accurate readings.[7]

The use of the antimony electrode has been objected to by Clement[8] because of its sensitivity to oxidizing and reducing agents, but his conclusions were not based on any tests in the mouth. His objections should not be discounted, because the antimony electrode is known to be sensitive not only to oxidizing and reducing agents but also to acid radicals of metaphosphoric, tartaric, citric, and oxalic acid,[9] heavy salt concentration, and copper and other metallic ions.[10] Therefore, the accuracy of the antimony electrode would appear to depend on whether interfering agents are present in the mouth in sufficient concentrations to yield erroneous results.

PROCEDURE AND RESULTS

The micro-antimony electrode used in this study and its method of application have been described in a previous paper.[1] A Beckman glass electrode, No. 290-1, was employed as a comparison instrument.

(A) *Intraoral pH Readings.*—When a buffer of pH 7 was applied on a tooth surface, the glass electrode gave a reading of 6.8 and the micro-antimony electrode a reading of 7.1. However, readings obtained on plaque and debris often disagreed to a greater extent. The antimony electrode gave higher readings than the glass electrode in most instances. The pH readings usually agreed within 0.4 to 0.05 pH unit, but differences of as much as 1 pH unit were observed.

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These differences suggested that interfering substances affected the antimony electrode. However, a number of factors made it difficult to conclude definitely that the antimony electrode was at fault. The accuracy of the glass electrode may be questioned because only part of its glass membrane comes in contact with the tooth surface during a measurement and the greater bulk of the glass electrode causes it to measure the pH of a much greater area than the micro-antimony electrode. The area contacted may result in some difference in pH readings, because it has been observed that pH obtained with the micro-antimony electrode may vary by as much as 1.4 pH units in two different areas on the same tooth, and by as much as 0.8 pH unit when the glass electrode was used. Thus, the pH obtained with the glass electrode probably represents the average pH of the contact area with the tooth, while the micro-antimony electrode determines the pH of a small spot within this area. In addition, the glass electrode contacts the external surface of the plaque while the micro-antimony electrode penetrates the soft material and measures the pH of deeper layers of plaque material. Because of the uncertainties involved in comparing glass and antimony electrode measurements in the mouth, further comparative tests were carried out in vitro.

(B) *In Vitro Tests.*—Glass and antimony electrode readings were taken on salivary sugar mixtures before and after incubation. Also, comparative pH readings and some titration curves were obtained on a number of organic acids and substances which may occur in the mouth, including lactic, pyruvix, tartaric, succinic, acetic, and thioglycolic acid, peroxide, and calcium and phosphate ions. Table I shows pH readings obtained with the glass and micro-antimony electrodes

TABLE I

pH READINGS OF SUGAR AND SALIVA MIXTURES WITH GLASS AND MICRO-ANTIMONY ELECTRODES

SAMPLE NO.	BEFORE INCUBATION		AFTER 17 HOURS' INCUBATION	
	GLASS	ANTIMONY	GLASS	ANTIMONY
1	7.4	7.5	4.2	4.4
2	7.9	7.9	4.2	4.5
3	7.8	7.6	3.9	4.2
4	7.6	7.7	4.1	4.5

in saliva-sugar mixtures before and after seventeen hours' incubation at 37° C. It will be noted that the antimony electrode consistently gave from 0.2 to 0.4 pH unit higher readings than the glass electrode. The same was true of saliva incubated for seventy-two hours. These differences were of smaller magnitude when the antimony electrode was standardized against the glass electrode in a 0.01N lactic acid buffer, thus suggesting that lactic acid might interfere with the accuracy of the antimony electrode. This was confirmed, as shown in Fig. 1, which gives titration curves of 0.5N sodium hydroxide against 0.6N lactic acid as obtained with glass and antimony electrodes. The pH readings were consistently 0.5 pH unit higher with the antimony electrode than with the glass

electrode. Similar divergent results were obtained with tartaric acid, but comparable results were obtained with acetic, pyruvic, succinic, and hydrochloric acid.

In further experiments, the effect of different concentrations of lactic acid, pyruvic acid, thioglycolic acid, and hydrogen peroxide was tested. The latter two substances were chosen in order to demonstrate the effect of reducing and oxidizing agents. The results are presented in Table II. It will be seen that lactic acid must be present in concentrations stronger than 0.01 normal to affect appreciably the antimony electrode. Thioglycolic acid and peroxide appear to be more powerful interfering agents, since concentrations as small as 0.0005M of thioglycolic acid slightly raised the antimony electrode reading, while peroxide had an opposite effect.

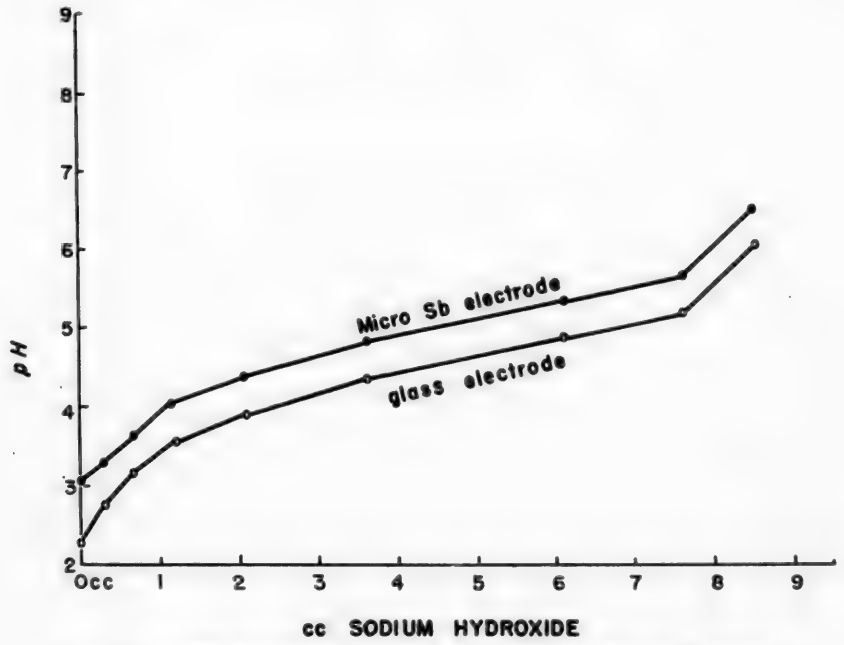

Fig. 1.—Titration curves of 0.6N lactic acid against 0.5N sodium hydroxide.

Calcium chloride did not affect the antimony electrode in concentrations up to 1M. Phosphate ions were also without appreciable effect.

According to these findings, the oxidizing and reducing agents tested must be present in concentrations greater than 0.001M to cause an error in the antimony electrode reading of 0.3 pH unit. Other interfering substances tested must be present in concentrations exceeding 0.01M to cause a similar effect. The concentration of any one of these substances is likely to be smaller in the mouth, but it is possible that they exert an accumulative effect.

It has already been shown that many substances may affect the accuracy of the antimony electrode. Since differences as great as 0.4 pH unit have been observed between glass and antimony electrode measurements of saliva, it appears that only approximately correct intraoral pH values may be obtained by means of the antimony electrode under certain conditions. However, since the micro-antimony electrode is the only known instrument capable of obtaining the pH values of otherwise inaccessible areas in the mouth, this electrode may be a valuable tool for the determination of the approximate range of pH change.

From the preceding discussion, it follows that in plaque tests concerned with the evaluation of acid-forming inhibitors, the antimony electrode is a suitable instrument only if its accuracy is proved to be unaffected by the test agent.

TABLE II

GLASS AND ANTIMONY ELECTRODE pH READINGS

APPROXIMATE CONCENTRATION (MOLAR)	LACTIC ACID		THIOGLYCOLIC ACID		PEROXIDE*	
	GLASS	ANTIMONY	GLASS	ANTIMONY	GLASS	ANTIMONY
0.6	1.94	2.48				
0.05	2.52	2.88	2.61	4.27		
0.01	2.96	3.07	2.96	4.05	3.98	2.80
0.001	3.56	3.52	3.55	3.80	4.00	3.55
0.0005			3.81	3.98	4.00	3.85
0.0001			4.64	4.65		

*In acetate buffer pH 4.

SUMMARY

1. The micro-antimony electrode frequently gave intraoral plaque readings which deviated from glass electrode readings by 0.3 pH unit or more, the maximal deviation observed being 1 pH unit. The differences in pH readings between the two instruments do not necessarily result from inaccuracy of the antimony electrode alone.

2. Observations of pH on saliva demonstrate that interfering agents may be present causing the micro-antimony electrode to give up to 0.4 pH unit higher readings than the glass electrode.

3. The effect of a number of interfering substances which may occur in the mouth has been studied at different concentrations.

4. Under certain conditions, intraoral pH measurements obtained with the micro-antimony electrode may be expected to be only approximately correct.

5. The micro-antimony electrode gives accurate in vitro pH readings in a number of acids and is well suited for certain electrometric micro titrations.

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RADIOAUTOGRAPHIC VISUALIZATION OF THE FORMATION AND FATE OF THE ORGANIC MATRIX OF DENTIN

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A RADIOAUTOGRAPHIC survey of the organs and tissues of very young rats injected with C^{14}-labeled bicarbonate revealed a widespread distribution of the radioelement.[1] The tissues of the developing incisors and molars displayed definite reactions, which were particularly intense in dentin. Evidence was presented to indicate that the sections of teeth used no longer contained minerals, even though they had not been systematically decalcified. Therefore, it was suggested that the radiocarbon had been incorporated into the dentinal matrix itself.[1]

A detailed study of this phenomenon was undertaken in the hope of extending our knowledge of the formation and fate of the organic components of dentin.

METHODS

The *solutions of C^{14}-labeled bicarbonate* used for injection were prepared from an alkaline solution of C^{14}-labeled sodium carbonate obtained from the Chalk River atomic pile. This material was partially neutralized by dropwise addition of 4 per cent acetic acid up to the phenolphthalein end point (pH = 8.3), a procedure which transforms most of the carbonate into bicarbonate. The volume of solution administered did not exceed 0.2 c.c. and was injected as a single subcutaneous dose.

The first group of animals (hereafter referred to as *the newborn group*) consisted of the ten newborn animals described in a previous paper.[1] After receiving either 20 or 40 microcuries of the labeled bicarbonate, four of these animals were sacrificed four hours after injection, three at twenty-four, and three at seventy-two hours after injection. For the present study, histologic sections of alcohol-formol-fixed jaws of these animals were decalcified by immersion in 5 per cent nitric acid for several minutes prior to being prepared for radioautography. The completeness of this decalcification procedure was assessed by means of the Von Kóssa technic for detection of calcium phosphate. In the undecalcified state, dentin stained black by this method, while the lack of staining of acid-treated sections was taken to indicate successful decalcification. Radioautography was carried out as indicated later in the article.

Six animals in a new group (hereafter referred to as the *three-day group*) were 3 days of age at the time of administration of the C^{14}-labeled sodium bicarbonate. Three animals of this group received 30 microcuries of the labeled

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material and were then sacrificed at intervals of one, three, and six days after injection. The remaining three animals also received an initial injection of 30 microcuries, but in addition received a second injection of 30 microcuries two days after the first. These three animals were then sacrificed at nine, twelve, and fifteen days following the first of the two injections.

Maxillae and mandibles were removed from these animals immediately after sacrifice, and were then fixed in alcohol-formol,[2] decalcified by the formate-citrate method,[3] dehydrated in dioxane, embedded in paraffin, and sectioned at 5 μ.

The sections of teeth used for radioautography were either stained with hematoxylin-eosin or left unstained. Control sections for histologic study were stained by hematoxylin-eosin, by the periodic acid-Schiff technic, or by toluidine blue for metachromasia. In the last case, preparations were immersed in a dilute alcoholic solution of the dye (0.1 per cent toluidine blue in 1 per cent ethyl alcohol) for approximately one-half hour, whereupon they were passed through single rapid changes of pure acetone, acetone-xylol (1:1), and three changes of pure xylol prior to mounting in Canada balsam.

Radioautography was carried out by the coating technic,[4] as more recently modified.[5] The emulsion utilized for this study was Eastman Kodak NTB3 emulsion, which was obtained either in the form of pellicles, to be melted before use, or in the form of a liquid which could be warmed and applied directly to the slides. Exposure times varied from five to ten months at –17° C. The slides were subjected to routine photographic development and fixation, followed by dehydration in xylol and mounting under Canada balsam.

The quantitative radioautographic technic of grain counting was applied in this study to determine the fate of labeled dentinal organic matrix. The rationale of this technic is based upon the fact that under conditions of low radioautographic exposure, the number of silver grains rendered developable by the ionizing radiations of C^{14} (or of any other beta-emitting radioisotope) is proportional to the amount of the C^{14} present in the source.[6] Thus, by counting the number of silver grains in the photographic emulsion lying over the tissue section in a developed radioautograph, it is possible to relate the count to the relative amount of isotope in the tissue.

Grain counting was carried out in the first lower molars of the animals of the three-day group. The radioautographs used for this purpose were prepared under identical conditions throughout; that is, all preparations were cut at the same thickness (5 μ), and were stained, coated, exposed, and developed simultaneously. Moreover, the preparations were allowed only a short exposure (five months) in order to maintain the condition of proportionalitiy of grain count to radioactivity.

The technic of counting grains was as follows: A reticle of two parallelly-oriented hairs was inserted into one ocular of a binocular microscope, so as to delineate an approximately rectangular strip of the field for counting. The strip extended across the center of the ocular field and was limited on its long sides by the hairs and on its ends by the margins of the ocular field. At the

magnification generally utilized (approximately 800 diameters), the width of the strip thus formed was approximately 30 μ, while its length was approximately 90 μ. The grain counts were obtained by enumerating all the grains visible in successive strips of dentinal matrix measuring approximately 30 by 90 μ, beginning at and lying parallel to the junction of the odontoblasts and predentin, and continuing to the dentinoenamel junction. After counting each strip, the slide under examination was moved in such a way that the next adjacent strip of matrix of the same dimensions was in the field provided by the reticle (Table I).

TABLE I

GRAIN COUNT OF ANTERIOR CUSP OF FIRST LOWER MOLAR (PEAK COUNTS* UNDERLINED)

DISTANCE FROM ODONTOBLASTS (μ)	ANIMALS SACRIFICED AT				
	3 DAYS (FIG. 5)	6 DAYS (FIG. 6)	9 DAYS (FIG. 7)	12 DAYS (FIG. 8)	15 DAYS (FIG. 9)
0-30	58	16	14	9	5
30-60	171	34	36	24	47
60-90	392	45	57	61	22
90-120	40	119	71	42	30
120-150	18	393	152	140	86
150-180		81	186	337	76
180-210			233	463	94
210-240			467	409	82
240-270			74	113	155
270-300					230
300-330					393
330-360					477
360-390					65
390-420					16
420-450					8
Total count	679	688	1,290	1,598	1,786
Peak count Total	563	512	1,038	1,349	1,255

*Peak counts represent the highest values obtained in a series of observations on radioautographic images of dentin matrix. Two peak count values were chosen arbitrarily in the case of singly injected rats (sacrificed 3 days and 6 days after injection), while four peak count values were chosen in animals which had been doubly injected (sacrificed 9, 12, and 15 days after injection).

In addition to these counts, several preliminary counts had been made on the same sections, utilizing an oil immersion objective which provided a total magnification of approximately 1,200 diameters. At this magnification, the distance between the two parallel hairs of the reticle in relation to the specimen was reduced to 20 μ, and the length of the strip was reduced correspondingly to approximately 60 μ. It was not possible to count all of the grains present in a given field under these conditions, since the depth of focus provided by the oil immersion objective was insufficient for this purpose. Hence, for each strip it was found necessary to count the grains in successive layers of the photographic emulsion. This was accomplished by focusing on and counting those grains most nearly adjacent to the tissue. Following this, the barrel of the microscope was raised 2 μ by the fine adjustment, and again those grains in focus were counted. Successive counts at 2 μ intervals were thus tallied

and totaled for the entire emulsion thickness in each strip, before continuing to the next adjacent strip. In this manner, the possibility of an error in counting was minimized, and the total counts for each adjacent strip of dentinal matrix were obtained in a comparable fashion. All counts were made on the anterior cusp of the first lower molars of these animals.

Some of the silver grains in the emulsion are not due to radiation, but are merely the background fog inherent in all photographic emulsions. To assess the extent of such background grains, several successive counts were made of grains lying in the photographic emulsion in areas devoid of tissue. These "background" counts were averaged, and were then subtracted from the counts obtained from the tissues.

RESULTS

Morphologic Observations.—The dentinal organ consists of the pulp, the odontoblasts, the predentin, and the dentin itself, which ends at the "junction"; that is, according to the area, dentinoenamel or dentinocemental junction. Predentin stained poorly and dentin deeply with hematoxylin-eosin (Fig. 1). Predentin reacted only slightly to the toluidine blue technic employed here (Fig. 16) and to the periodic acid-Schiff technic (Fig. 17). Dentin, on the other hand, was intensely metachromatic (Fig. 16) and stained readily with the periodic acid-Schiff technic (Fig. 17).

In the *newborn group,* the incisor and molar teeth at four hours after injection showed a precisely localized and relatively intense radioautographic reaction (that is, accumulation of silver grains) over the region of predentin immediately adjacent to the odontoblasts (Fig. 1). No reaction was observed over the dentin already present in the teeth at this interval (note unreactive dentin [*D*] in Fig. 1). The latter observation was further emphasized by the fact that the reaction of predentin was clearly visualized even in areas where no dentin had yet been formed (Fig. 2). Little or no reaction was observed over the odontoblasts themselves.

By twenty-four hours, little change in the intensity of this reaction was observed, but its distribution was slightly altered. Some reaction was present in predentin, while most of it was in that portion of dentin in apposition to the predentin. In some of the areas where only predentin had been present at four hours, some reactive dentin had now appeared.

At seventy-two hours, the predentin was unreactive, while many silver grains were seen over the dentin proper. No odontoblastic reaction was seen at this or any later time interval.

It may therefore be concluded that the reactive material was present exclusively in the predentin at four hours and in the dentin at seventy-two hours after injection. These results are interpreted as indicating that the predentin and dentin formed during the latter part of the three-day period following injection of C^{14} contained only small amounts of the isotope, since the level of circulating labeled bicarbonate had by that time fallen too low to supply a significant amount of radioactivity to the synthesis of these materials.

The radioautographic reactions of teeth seen in the *three-day group* of animals at the early time intervals presented essentially the same features as did those of the newborn group. At twenty-four hours after injection, the most intense radioautographic images in the molars occupied a position similar to that seen in the newborn group at the same interval, that is, in the inner layers of dentin and in the juxtaposed outer layers of predentin (Fig. 10). This reaction extended throughout the complete circumference of the molar bud (Fig. 3). Since the area of predentin immediately in contact with the odontoblasts contained very few granules (Fig. 10), the predentin laid down during the latter part of the twenty-four-hour period between C^{14} injection and sacrifice contained only a small amount of radioactivity. In other words, most of the labeled material had been deposited during the earlier part of this twenty-four-hour period.

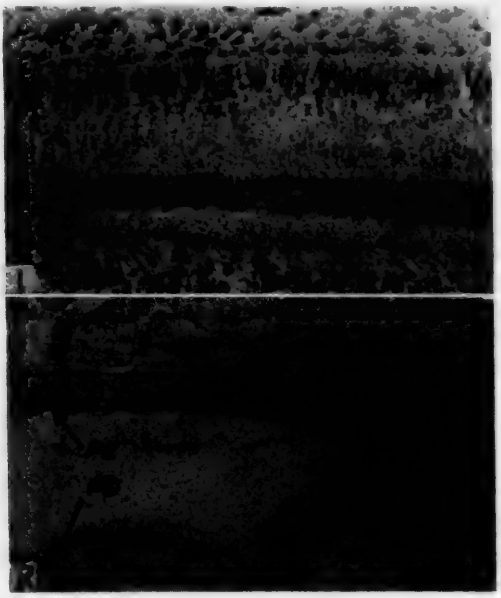


Fig. 1.—Coated radioautograph of a portion of an incisor tooth taken from a rat injected at birth with 40 microcuries of C^{14}-bicarbonate and sacrificed four hours later. The section was stained with hematoxylin and eosin prior to coating with Eastman Kodak NTB3 emulsion. (×180.) *A*, Ameloblasts; *PE*, pre-enamel; *D*, dentin; *PD*, predentin; *O*, odontoblasts. The dentin, which appears here as a dark band, shows no significant radioautographic reaction. The predentin, which appears light, shows a radioautographic reaction in the area close to the tips of the odontoblasts. (A slight reaction is present in pre-enamel.)

Fig. 2.—Coated radioautograph of the root region of an incisor tooth taken from the same animal as in Fig. 1, but for which the preparation was exposed for a longer time. The abbreviations are the same as in Fig. 1. In this area no dentin has yet formed. Even so, the predentin shows an intense reaction. On the left of the figure the predentin broke away from the pre-enamel during the technical procedure. This artefact makes it all the more evident that the reaction is in predentin.

By three days after injection, the band of reaction was strictly limited to the dentin, where it was seen as a definite and discrete line about midway between the odontoblasts and the dentinoenamel junction (Fig. 11). Comparison of this pattern with that seen at twenty-four hours indicated that the distance

between the line of radioautographic reaction and the dentinoenamel junction had not changed over the intervening forty-eight-hour period; that is, an area of unreactive dentin of the same approximate thickness was observed between the reactive line and the dentinoenamel junction at both twenty-four and seventy-two hours. Since most of the labeled material was deposited during the earlier part of the twenty-four-hour period following C^{14} injection, a preparation such as that depicted in Fig. 4 may be interpreted with precision: according to whether the dentin is present outside or inside the dark band (Fig. 4), it was laid down, respectively, before or after the earlier part of the twenty-four-hour period following C^{14} injection.

By six days after injection (Fig. 12), the band of autographic reaction in the dentin remained clear cut, and its intensity appeared the same as at the earlier intervals. The distance between the line and the dentinoenamel junction on the cusp crown also remained approximately the same as that seen at the earlier times. More new dentin had been formed by the odontoblastic layer, however, so the distance between the line and the odontoblasts had increased further. The reaction line followed the contours of the cusps (Fig. 5), lying at some distance from the dentinoenamel junction at the crowns, but lying against the junction at the bases of the cusps. By this time, the roots of the molars had lengthened, and naturally the new dentin deposited near the tips of the roots was unlabeled.

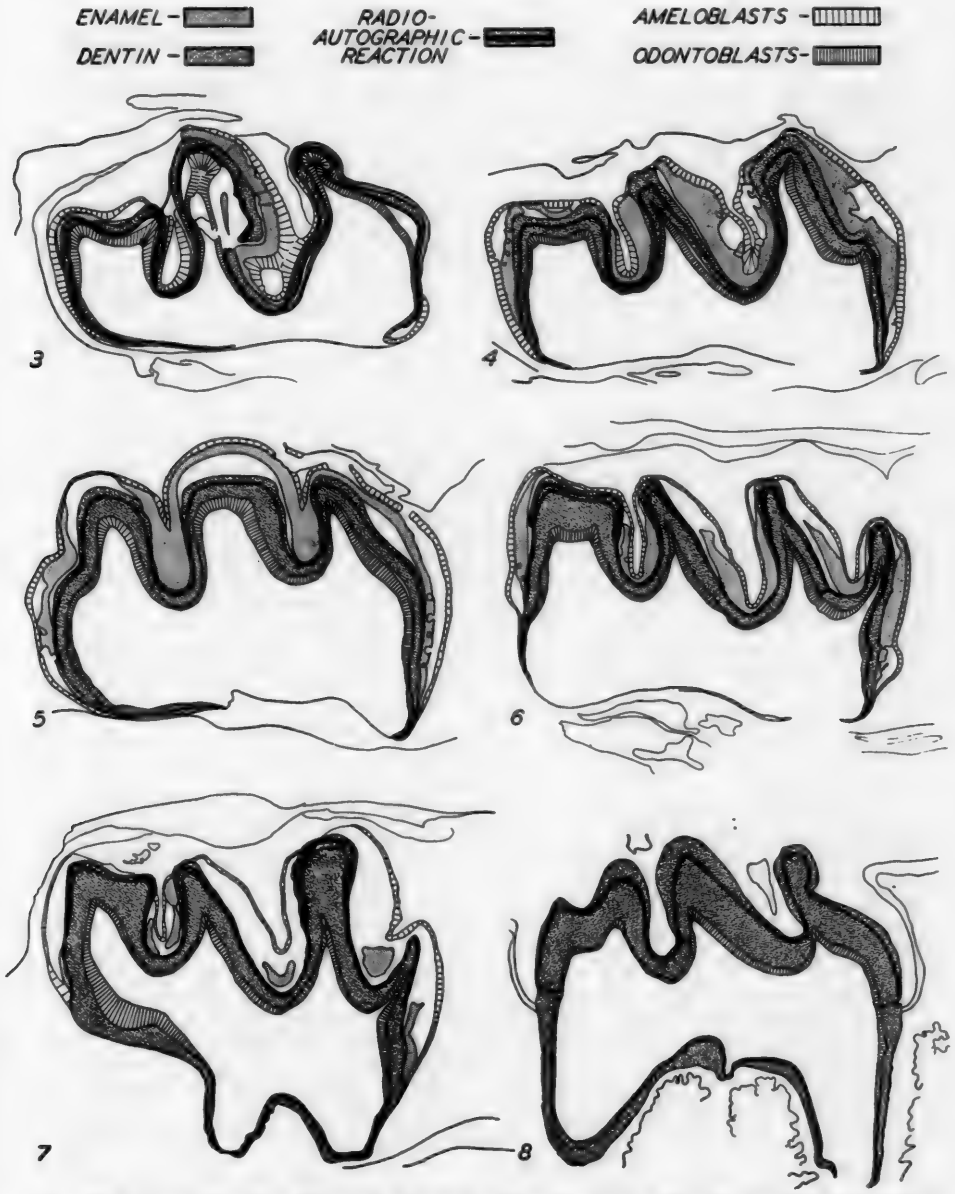
The animal sacrificed at nine days after injection had received a second injection of C^{14}-labeled bicarbonate two days after the first, and indeed the radioautographs showed a double line of reaction in the dentin (Figs. 9 and 13). Again, the intensity of each of these lines was similar to that of the lines seen at the earlier intervals. The outer and inner lines, resulting from the first and second injections, respectively, were separated by a layer of unreactive dentin which was clearly seen only in the region of the crowns of the cusps. In the area of the roots, the lines were fused (Fig. 6).

The radioautographic images in the molars of the doubly injected animal sacrificed at twelve days were essentially the same as in the nine-day animal (Figs. 7 and 14).

In the animal sacrificed at fifteen days, the molar tooth had erupted. The distinction between two reaction lines was not clear in this case (Figs. 8 and 15). The distance between the line and the dentinoenamel junction was approximately the same here (Fig. 8) as in the animals sacrificed at one, three, and six days (Figs. 3, 4, and 5), but was somewhat greater than at nine and twelve days (Figs. 6 and 7), possibly as the result of individual variations within the group of animals.

The reactions of incisor teeth in the three-day group of animals seemed to be analogous to those just described in the molars, within the limits of comparison of the two types of teeth.

Grain Counting.—To confirm the observation that with time there seemed to be little or no change in the intensity of the autographic reactions visualized in



Figs. 3-8.—Projection diagrams of coated radioautographs of the first molars taken from rats injected with C^{14}-bicarbonate when 3 days old and sacrificed at the following intervals after injection: One day (Fig. 3); three days (Fig. 4); six days (Fig. 5); nine days (Fig. 6); twelve days (Fig. 7); fifteen days (Fig. 8). In addition, the animals sacrificed at the three last intervals received a second injection of C^{14}-bicarbonate when 5 days old.

the dentinal matrix, it was decided to make a quantitative study of the autographic reactions in the molars of the three-day group.

To show the feasibility of the method, the silver grains were counted over strips of dentinal matrix at the anterior cusp crown of the first lower molar at a magnification of 1,200 diameters (oil immersion). The results obtained in a nine-day animal are illustrated in Fig. 9, in which the peaks in the graph of the counts correspond to the two bands of reaction seen in the photograph below it.

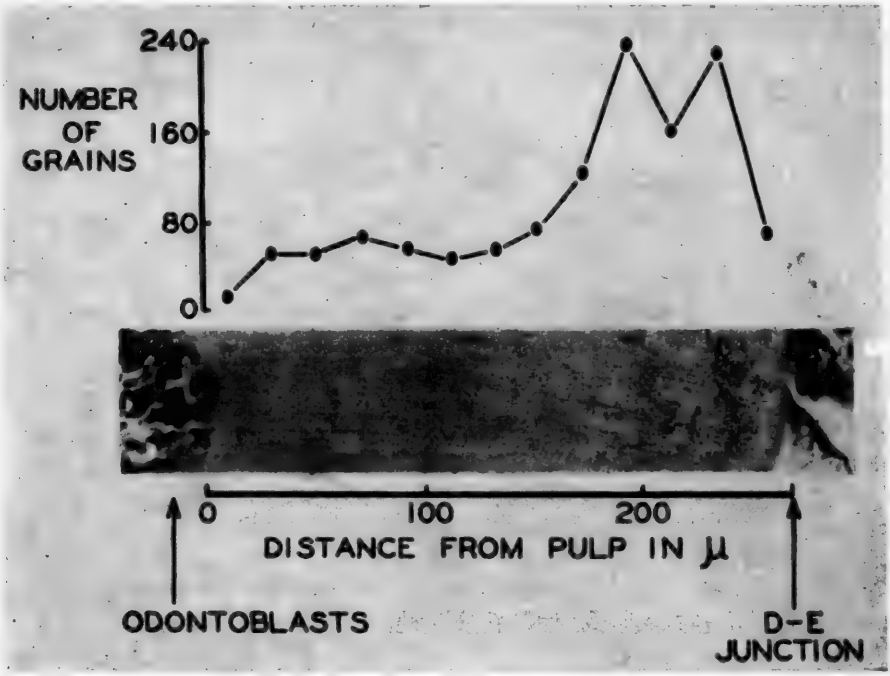
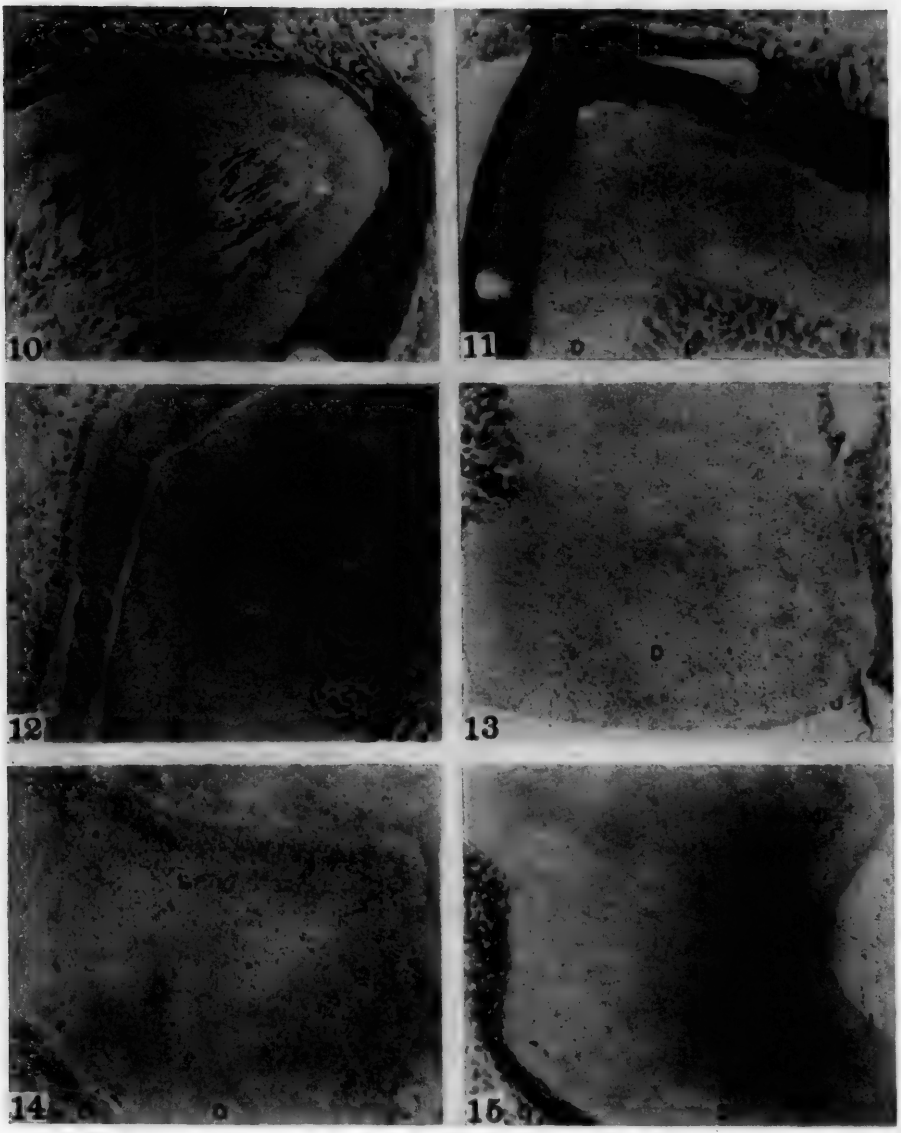


Fig. 9.—Grain count in the dentin from the region of the odontoblasts to junction in an animal injected twice with C^{14}-bicarbonate, namely, at three and five days after birth. The animal was sacrificed nine days after the first injection. The silver grains were counted at a magnification of 1,200 diameters. The two peaks seen in the graph correspond to the two bands of reaction seen in the dentin.

The thickness of some of the other preparations made it impossible to examine them all at 1,200 diameters. Systematic counts were, therefore, carried out at a magnification of 800 diameters, utilizing radioauotgraphic preparations from the animals sacrificed at three, six, nine, twelve, and fifteen days. (Comparable sections from the animal sacrificed at one day were not available for study.) All the silver grains present over strips of dentinal matrix on the anterior cusp crown of the first lower molar from each animal were counted. From these counts, the background count was subtracted to yield the data presented in Table I. (The background values obtained were, respectively: three days, 66; six days, 64; nine days, 56; twelve days, 70; and fifteen days, 79.)

The results obtained at 800 diameters did not make it possible to distinguish the two peaks seen at 1,200 diameters (Fig. 9). In order to obtain a measure



Figs. 10-15.—High magnification of the anterior cusp crown of the first lower molar from the animals of the three-day group. After injection of C^{14}-bicarbonate when the animals were 3 days old, they were sacrificed at: one day (Fig. 10); three days (Fig. 11); six days (Fig. 12); nine days (Fig. 13); twelve days (Fig. 14); fifteen days (Fig. 15). (The last three animals received a second injection two days after the first.)

of the intensity of the reaction lines seen at the various intervals, the two greatest values were noted and totaled for each animal which had received only one injection of the isotope. In the case of the doubly injected animals, the four highest values were used for this purpose. These values, probably the best ones to use for comparative purposes, are underlined in the body of Table I, and their total is also underlined as the "peak count total."

Examination of Table I reveals that whether the "total count" or the "peak count total" is considered, the quantity of silver grains deposited in the photographic emulsion of the radioautographs was approximately the same in the animals given a single injection (three and six days) and about double that value in the animals given two injections (nine, twelve, and fifteen days). It is clear that there was no decrease with time in the amount of labeled material deposited in the matrix.

DISCUSSION

The presence of radioautographic reactions in the matrix of the dentin in growing rats injected with C^{14} bicarbonate (Figs. 10 through 15) is a finding of significance in regard to both the growth pattern and the composition of the matrix.

Growth Pattern of Dentinal Matrix.—As early as four hours after injection of labeled bicarbonate, radioactivity appeared in the portion of the predentin juxtaposed to the apices of the odontoblasts (Figs. 1 and 2). The radioactive band seen in the sections was due to the presence of a layer of C^{14}-labeled predentin matrix located near the odontoblasts. Apparently, some of the injected C^{14} had been utilized for the elaboration of the predentin matrix deposited during this four-hour period.

At twenty-four hours after injection, the layer of labeled matrix had been displaced outward due to the continuing apposition of new, unlabeled layers of predentin. Furthermore, the labeled layer, initially predentinal, was changing into dentinal matrix (Fig. 10).

At three days, and at later times after the injection of labeled bicarbonate, the growth of dentin was visualized by a progressive increase in the distance between the initial labeled band and the odontoblasts (Figs. 10 through 15). This distance increased very rapidly at first (Figs. 10, 11, and 12), and more slowly at the longer time intervals (Figs. 13, 14, and 15). Thus, although these conclusions are based on only six animals, it appears that the growth rate of dentinal matrix was rapid in animals during the first ten days of life, after which it slowed down.

Examination of the growth of the various regions of the molar tooth (Figs. 3 through 8) illustrated several points already observed by other methods,[7, 8] namely, the continued root growth, the progressive encroachment of dentin into the pulp cavity—a process balanced by the expansion of this cavity into the root region concomitant to root growth—and, in doubly injected rats, the

more rapid apposition of dentinal matrix in the crowns of the cusps than in the roots, as shown by the distinctness of the two lines of reaction in the former but not in the latter area (Figs. 6 and 7).

The results of this work serve to emphasize two properties of the dentinal matrix, namely, its appositional mode of formation and its stability. The doubly injected animals clearly show the appositional deposition of matrix (Figs. 6 and 7). In addition, the absence of silver grains in the area between the reactive band and the dentinoenamel junction indicates that no interstitial deposition of material occurs in formed matrix. The presence of a small number of silver grains in the matrix laid down for some time after injection (Table I) does not disprove that dentin grows appositionally, since the grains are few and presumably reflect a progressive decrease in the blood levels of C^{14} utilizable for matrix formation.

The dentinal matrix, as indicated by its labeled portion, is a stable substance. The grain counts through the fifteen days after injection showed no decrease in the amount of labeled material (Table I). Such stability of the matrix is all the more impressive since a survey of the distribution of C^{14} bicarbonate in very young rat tissues showed a generalized and almost complete decrease in the radioautographic intensity of organs and tissues with time, a phenomenon attributed to turnover of structural components.[1] Thus the dentinal matrix stands out as one of the most stable tissues of the growing body.

Mineral elements of dentin also are formed appositionally, as shown by P^{32} radioautography[9] and other methods.[7, 8] However, the exactness of their appositional deposition as shown by either P^{32} or Ca^{45} radioautography is less striking. In fact, some interstitial deposition of minerals throughout the thickness of the dentin, even occlusally to the main band of reaction, is usual in young animals. Newborn rats will often show no definable band at all but rather a diffuse reaction. In addition, a reaction band often becomes less precise with time,[2] although it always persists in some form.[10] It is indeed believed that mechanical influence may produce a rearrangement of dentinal crystals.[11] Therefore, it is concluded that the matrix is deposited in a more regular manner and is more stable than the mineral components of dentin.

Nature of the Labeled Substances in the Dentinal Matrix.—It has been pointed out that the labeled substances detected by radioautography in organs and tissues of animals given C^{14}-bicarbonate had to be substances of large molecular weight, such as proteins and/or mucopolysaccharides.[1] This is likewise true of the labeled component(s) of the dentinal matrix. It is known that this matrix consists of a protein framework, presumably a collagen, associated with carbohydrate material, some of which may be detected by the metachromatic staining of the matrix with toluidine blue and by its reactivity to the periodic acid-Schiff technic.[12-14] In contrast, the predentinal matrix stained only slightly when the latter two technics were used, as shown in Figs. 16 and 17. Thus, the predentin seems to consist mainly of collagenous material.[14] Deposition of reactive carbohydrate material must have predominated at the predentinodentinal junction. In fact, differences in the content of these reactive carbohydrates could account for the observed differences between the predentin and dentin.

If this is indeed the case, the labeled material appearing in predentin after C^{14}-bicarbonate injection could be part of the collagen framework. Since the radioautographs showed no apparent change in this labeled material at the time of the transformation of predentin into dentin, the collagen of the first zone would not differ from that of the other zone, except for the addition of mucopolysaccharide(s) at the junction.

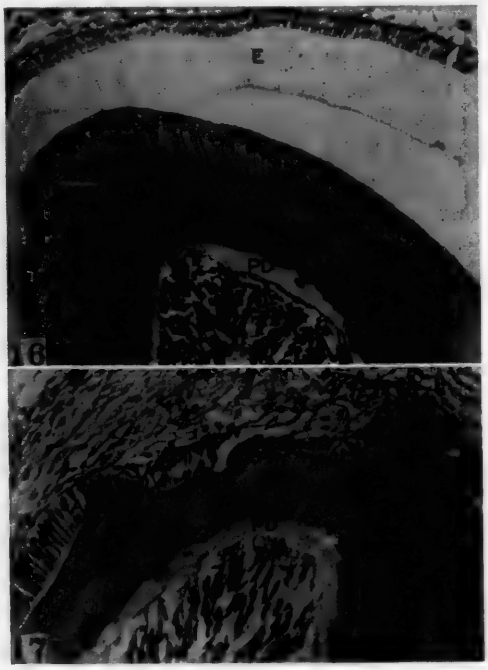


Fig. 16.—Toluidine blue staining of molar teeth of 3-day-old rat. Abbreviations as in Fig. 1. The nuclei of the ameloblasts and odontoblasts stain blue. The dentin is a deep violet and the predentin a very light pink.

Fig. 17.—Periodic acid-Schiff staining of molar tooth of 3-day-old rat. Counterstaining with hematoxylin. (This section was taken from the center of the cusp and, therefore, shows at the apex an area devoid of enamel.) The dentin stains deeply and the predentin little or not at all with the periodic acid-Schiff technic.

It is known that calcium phosphate crystals are absent in predentin and are abundant in dentin, and also that radioautographs show the deposition of Ca^{45} and P^{32} in that area of the dentin which is in apposition to predentin,[10] that is, where large amounts of carbohydrate materials also appear. It is tempting to conclude that the carbohydrate materials may be necessary for the deposition of the dentin crystals and may, in fact, bind these crystals to the collagen framework.

SUMMARY

Sodium bicarbonate labeled with C^{14} was administered to newborn and 3-day-old albino rats, whereupon these animals were sacrificed at intervals of from four hours to fifteen days after injection. Radioautographs of their teeth were prepared after decalcification.

The radiocarbon of bicarbonate is incorporated into the organic matrix of the dentinal organ. At the earliest time intervals studied, the labeled matrix lies in the predentinal area. But at later intervals, the reactive zone is farther and farther and farther away from the odontoblasts as new, nonlabeled predentinal matrix is laid down. In the process, the labeled matrix of predentin becomes dentin matrix proper.

Counts of the individual silver grains constituting the radioautographic image of the matrix show no decrease in its intensity during the fifteen-day period studied. Thus, the labeled matrix and, by inference the whole matrix of dentin, is a very stable substance (in contrast to C^{14}-labeled substances formed in other organs).

The formation of dentin may be considered to occur in three steps: (1) the formation of a matrix framework, presumably a collagen, which first appears as predentin at the apices of the odontoblasts (this collagenous material is presumably the substance which is traced by means of C^{14}); (2) the addition of large amounts of carbohydrate material to the framework of the matrix, changing it from predentin to dentin; and (3) the appearance of calcium phosphate crystals in the matrix of dentin.

Because of their simultaneous appearance at the predentinodentinal junction, it is suggested that the crystals may be bound to the collagen matrix of dentin by means of the carbohydrate material.

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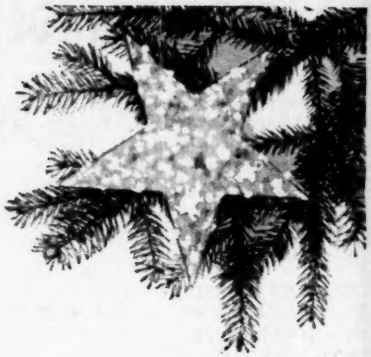

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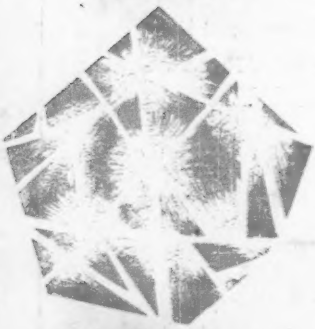

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